MEMOIRS
OF THE
FACULTY OF FISHERIES
HOKKAIDO UNIVERSITY

Vol. 9, No. 1

THE FACULTY OF FISHERIES, HOKKAIDO UNIVERSITY
HAKODATE, JAPAN
1961

MEMOIRS
OF THE
FACULTY OF FISHERIES
HOKKAIDO UNIVERSITY

Vol. 9, No. 1

THE FACULTY OF FISHERIES, HOKKAIDO UNIVERSITY
HAKODATE, JAPAN
1961

TAXONOMIC AND ECOLOGICAL STUDIES OF THE GENUS *HYPOMESUS* OF JAPAN

KEIKICHI HAMADA

TAXONOMIC AND ECOLOGICAL STUDIES OF THE GENUS *HYPOMESUS* OF JAPAN

KEIKICHI HAMADA

Faculty of Fisheries, Hokkaido University, Hakodate, Japan

Contents

I. Introduction

Hypomesus olidus and *Hypomesus japonicus* are known, at present, as fishes belonging to Gen. *Hypomesus* in Japan. These two species are closely related to *Oncorhynchus masou, Oncorhynchus keta, Osmerus dentex* and *Plecoglossus altivelis* inhabiting Japan. *H. olidus* is distributed in the northern Japan. The larva lives in fresh waters, then descends into the sea, where it grows and matures, and at last ascends to spawn in the fresh waters, as do also *O. keta* and *O. masou.* This species passes into a land-locked form without difficulty. Accordingly they are transplanted artificially to lakes and ponds in almost all regions. Transplantation is a great success in the artificial propagations of *H. olidus.* The writer thinks however that the success was brought about rather by the empirical application of the habits of *H. olidus,* which is easily land-locked, than as a result of research based on biology. The fittest ways and means of protection and culture of the aquatic animals may be found through biological investigations.

There are still different opinions on the classification of Gen. *Hypomesus,* especially on *Hypomesus olidus* and *H. japonicus.* Berg, L. S. (1932) regarded *H. japonicus* as a synonym of *H. olidus,* while Hubbs, C. L. (1925) and Hamada, K. (1953) separated *H. japonicus* from *H. olidus.* Taranetz, A. (1936) and Schmidt, P. U. (1950) maintain their opinions that *H. japonicus* is the synonym of *H. pretiosus.* According to Hamada, K. (1953), *H. japonicus* was never found in the fresh waters at any season of year in spite of his detailed investigation. However, there are a few authors who expressed some doubts on the classification of *Hypomesus.* One of these doubts may be caused by the reason of lack of detailed observation on the spawning habits of *H. japonicus,* though many authors consider merely that *H. japonicus* spawns on the algae or marine plants in the surf. Another one of the doubts may be caused by the fact that

the number of vertebrae may vary. The species may be really understood through the ecological studies in conjunction with the morphological.

It is being undertaken to stock the sea with larvae of *H. olidus* as a means of the culture of this species. However, there are some doubts on the effect of the stocking. Increase of the population of *H. olidus* along the coast is not recognized, and the population of *H. japonicus*, while should not relate to *H. olidus* at all, is increasing. The writer treated *H. japonicus* independently from *H. olidus* as a different species though a few authors regard *H. japonicus* as a synonym of *H. olidus*. It may be caused by the spawning habit of *H. japonicus* that the population of the smelt is increasing on the coast of Uchi-ura Bay in recent years. If *H. japonicus* is identified with *H. olidus*, the increase of the population of *H. japonicus* will be considered as the effect of the artificial culture of *H. olidus*. The writer wishes to offer some explanation of this problem.

As stated above, the problems in the taxonomy relate to the fishery, and the object of the taxonomy is not be attained completely by only a comparative study of the characters of the body form. Species may be made clear more exactly by comparisons and considerations of thc life. The writer drew conclusions for the above problems through the taxonomic and ecological studies.

The creation of a new species or a new genus of sexually reproducing animals, through the branching of an evolutionary line, requires, that a portion of the original common stock become isolated (Neave, F. 1958). According to Dobzhansky, Th. (1951), the kind of isolation which seems to prevent interbreeding between existing species can be classified as: (1) geographical, (2) ecological, (3) temporal, (4) sexual, (5) mechanical and gametophytic isolation.

The author found out, through his study, some forms of the pond smelt which are without mingling of individuals for purposes of reproduction by the different habit or the difference of the breeding season. Neave, F. (1958) maintains his opinion, cited from Lack, D. (1949), that ecological divergence is often the result rather than the cause of speciation. But the ecological divergence found in the pond smelt aroused the considerable attention of the author, as the important mechanism which originates ecological isolation or the temporal isolation. It is maintained, in the present paper, that the ecological divergence is important as the cause of race formation or species formation.

First of all, the writer wishes to express his sincere thanks to Prof. Saburo Saito for his encouragement and guidance. He is much indebted to Prof. Tetsuwo Inukai for his valuable suggestions and critical reading of the manuscript, and he is also sincerely grateful to Prof. Toichi Uchida for his valuable counsel. He wishes also to express his heartfelt gratitude to Asst. Prof. Hidejiro Niiyama for valuable discussions and his advice. He is very glad to record gratitude to the persons who were so kind as to give him the valuable specimens. He is especially indebted to Dr. Yoshikazu Shiraishi of the Freshwater Fisheries Research Laboratoy who gave him the specimens, and to Dr. Ryuhei Sato of Tohoku University for kindness afforded to the writer in re the collection of the specimens; further, appreciation is expressed to Mr. S. Tanaka, Mr. E. Ito, Mr. H. Sato, Mr. Y. Ogawa, Mr. K. Ito, Mr. S. Abe and Mr. T. Kobayashi.

II. Material and Method

Information about the samples used in this study is recorded in tables 1 to 5 and o-I. The samples shown in table 1 are the fish obtained from the river Ishikari. Table 2 shows the samples obtained from Lake Abashiri and adjacent waters, Lake Tofutsu and the coast of Abuta, Uchi-ura Bay. Table 3 regards the samples from Lake Ishikari-Furukawa (old water-way of the river Ishikari) and Lake Onuma. Table 4 describes the samples collected in Lakes Kogawara, Suwa, Yunoko, Kasumigaura, Kawaguchi, Yamanaka, Kizaki and Ikeda. The samples shown in table 5 were obtained from the coasts of Mori, Hakodate, Muroran and Lake On-neto (a salt lake). Table o-I shows the samples from the bay of Oshoro. Of the samples listed above, the specimens shown in tables 1, 2, 3, 4 and table o-I are *H. olidus* with the exception of samples 16, 17a, 17c, 18a and 19a which are *H. sakhalinus*. All specimens of table 5 are *H. japonicus*. All numbers in tables and text-figures are those of samples as shown in tables 1 to 5 and o-I. Samples 1 and 3 are *H. olidus* ascending into the Ishikari River, while sample 2 of *H. olidus* taken from the lower part of the river Ishikari is a fish descending into the sea after spawning. Among the specimens of table 2, samples 5, 10 and 11 are *H. olidus* in just spawning period, while all others are the fish in the period of the growth. In table 3, samples 18a and 18b are fish in the growth period, and the others are in the period of the spawning. In table 4, samples 24 and 25 are fish in the period of growth and the others are in the period of spawning or a little after the spawning, while the others are in the period of the growth. Some samples are however omitted from the tables and the figures, for their data had been already reported in other papers, or the measurements and counts on some portions were not carried out. Because of the partial breakage of the specimens, the numbers of specimens showed in tables 1 to 5 and o-I do not agree, in some cases, with the numbers of the specimens shown in other tables. Tables o-I to o-III present the data of the pond smelt obtained from Oshoro Bay.

The samples used for the observation of the embryonic development are the eggs of 22 individuals of *H. olidus*, 45 to 83.1 mm in body length, taken from Lake Onuma on April 8, 1960, and 12 individuals of *H. sakhalinus*, 61 to 108.5 mm in body length, taken from Lake Ishikari-Furukawa on April 19, 1960.

All specimens were preserved in about 10% formalin. The methods of measurement and the count used in the description are as follows: body length is the distance from tip of lower jaw to midbase of caudal fin. Head length is the distance from tip of lower jaw to end of operculum. Depth is the greatest depth of body. Length of maxillary is the distance from tip of snout to rear edge of maxillary. Snout is the distance from tip of snout to front of eye. Diameter of orbit is the length of diameter in parallel to vertebrae. Interorbital width is the least fleshy distance between eyes. Depth of caudal peduncle is the least dorsoventral distance. The number of fin rays includes branched rays, unbranched rays and rudiments. In counting of fin rays, each ray with a separate base was counted as a single ray. The number of gill-rakers on the first arch includes rudiments. The number of vertebrae includes urostyle.

Length of pectoral fin is the length from base of the first ray to tip of fin. Distance from the pectoral fin to ventral is the distance from base of the first pectoral ray to that of the first ventral ray, and expressed by the mark p-v.

In the present paper, "0 age fish" means the fish which is less than twelve months, 1 age fish is the fish which is twelve months and more, 2 age fish is the fish which is two years and more, and so on.

III. Taxonomic Study on Samples from Japan

A) *Hypomesus olidus* obtained from the river Ishikari (Table 1, samples 1, 2 and 3, and table o-I, samples o-1 to o-6)

Table 1. *H. olidus* obtained from the river Ishikari

No. of Sample	Locality	Date	Species	Number of Individuals		Body Length (mm)
				Male	Female	
1	River Ishikari	May 27, 1949	*H. olidus*	53	58	114—90
2	River Ishikari	May 25, 1956	*H. olidus*	43	57	125.4—78.5
3	River Ishikari	May 19, 1957	*H. olidus*	47	53	113—86.9

Table o-I. *H. olidus* obtained from Oshoro Bay

No. of Sample	Date	Number of Individuals			Body Length (mm)
		Male	Female	Indistinct-ness	
0-1	Jul. 31, 1952	31	21	49	42.8—35.7
0-2	Sept. 3, 1952	1	7		70.5—64
0-3	Oct. 3, 1952	3	7		83—75
0-4	May 8, 1953	1			101
0-5	Aug. 4, 1953			1	35.5
0-6	Aug. 19, 1953	6	5	90	58—49

a) The school of *H. olidus* ascends to spawn in the river Ishikari from the sea in the middle or toward the end of May (Table 1). This school is separated as one form (or race) with a difference in number of vertebrae and difference in ecology from other pond smelt. The figure a shown in Pl. I, is one specimen of the sample. Taxonomic characteristics are as follows:

Body length 102.4 mm, male. May 27, 1949.

Depth 19.6%; head 22.1% of body length. Maxillary 40% of head, with minute canine like teeth. Snout 29%; orbit 22.1%; interorbital width 21% of head, and 96% of length of snout. Depth of caudal peduncle 38.6% of head. D. 9; A. 17; P. 13; V. 8, insertion being in advance of the vertical from the dorsal origin; Lat. l. 58; vertebrae 57; pyloric caeca 5 in number. Lateral line incomplete, external openings 14; pectoral length 55.9% of p-v. Color lighter

Table o-II. Frequencies of the number of dorsal rays of *H. olidus* obtained from Oshoro Bay

No. of Sample	Number of Dorsal Rays			
	9	10	11	12
0-1	1	30	61	9
0-2		1	6	1
0-3		2	8	
0-4		1		
0-5	1			

Table o-III. Frequencies of the number of anal rays of *H. olidus* obtained from Oshoro Bay

No. of Sample	Number of Anal Rays					
	15	16	17	18	19	20
0-1	4	24	39	31	3	
0-2			3	1	3	1
0-3		1	4	5		
0-4			1			
0-5		1				

than *H. olidus* obtained from other waters.

The descriptions of samples 1, 2 and 3 are as follows:

Body length 78.5 to 125.4 mm.

D. 9 to 12; A. 15 to 20; P. 12 to 14; vertebrae 54 to 58 in number, mostly 57 or 58; gill-rakers 31 to 37, mostly 34; pyloric caeca 4 to 6 in number; snout longer than interorbital width; head length 19 to 24% of body, diameter of orbit 19 to 24% of head, rarely larger; maxillary 38 to 44% of head, commonly 40 to 42%.

b) *Hypomesus olidus* obtained from Oshoro Bay (Table o-I)

These specimens are the pond smelt which ascends to spawn in the river Ishikari in the subsequent spring.

Body length 35.7 to 101 mm. D. 9 to 12 rays; A. 15 to 20 rays; vertebrae

Table 2. *H. olidus* obtained from Lake Abashiri, a river flowing into Lake Abashiri, the coast of Abashiri, Lake Tofutsu and the coast of Abuta

No. of Sample	Locality	Date	Species	Number of Individuals		Body Length (mm)
				Male	Female	
4	Lake Abashiri	Oct. 23, 1947	*H. olidus*	12	8+1	135—57
5	Lake Abashiri	Apr. 24, 1948	*H. olidus*	12	41	116—63
6	Lake Abashiri	Oct. 26, 1949	*H. olidus*	79	71	91—45
7	Lake Abashiri	Oct. 27, 1952	*H. olidus*	53	50	74—39
8	Lake Abashiri	Sept. 29, 1953	*H. olidus*	42	59	111—34
9	Lake Abashiri	Nov. 3, 1953	*H. olidus*	52	59	133—39.3
10	river flowing into L. Abash.	Apr. 26, 1955	*H. olidus*	62	35	92.3—50.9
11	river flowing into L. Abash.	Apr. 27, 1955	*H. olidus*	47	23	108.9—53.5
12	Port Abashiri	Oct. 22, 1949	*H. olidus*	56	93	85—58
13	Coast of Abashiri	Sept. 21, 1950	*H. olidus*	40	17	80—57
14	Lake Tofutsu	Sept. 29, 1953	*H. olidus*	34	25	118—47
15	Coast of Abuta	Nov. 23, 1952	*H. olidus*	52	45	107—61

54 to 59 in number, commonly 56 to 58.

B) *Hypomesus olidus* obtained from Lake Abashiri and adjacent waters, and two other waters (Table 2)

a) *Hypomesus olidus* obtained from Lake Abashiri and a flowing river (Table 2, samples 4 to 11)

The taxonomic characteristics of the specimens are as follows:

Body length 34 to 135 mm. D. 9 to 11; A. 14 to 19, 12 in one specimen; P. 11 to 15; vertebrae 53 to 59 in number, commonly 55 or 56; length of snout longer than the interorbital width, rarely shorter; pyloric caeca 4 to 6 in number, rarely 7; gill-rakers 31 to 37 in number, rarely less; head length 22 to 26% of body, 30% in one specimen; diameter of orbit 21 to 29% of head, rarely smaller; maxillary 37 to 50% of head, commonly 40 to 45%.

b) *Hypomesus olidus* obtained from the port of Abashiri and the adjacent coast (Table 2, samples 12 and 13)

Body length 55 to 89 mm.

D. 9 to 11; A. 14 to 19; P. 11 to 14; vertebrae 54 to 58, generally 56; length of snout greater than interorbital width, or equal; gill-rakers 31 to 37 in number.

c) *Hypomesus olidus* obtained from Lake Tofutsu (Table 2, sample 14)

Body length 47 to 118 mm. Not measured in detail. Number of vertebrae 54 to 58, mostly 56.

d) *Hypomesus olidus* obtained from the coast of Abuta (Table 2, sample 15)

Body length 45 to 52 mm.

D. 9 to 14; A. 14 to 17; P. not counted; vertebrae 53 to 58, usually 56.

C) *Hypomesus sakhalinus*, **Ishikari-wakasagi**,[1] was reported by the writer from Sakhalin, and he also found it newly from Lake Ishikari-Furukawa in Hokkaido, Japan. *H. olidus* obtained from Lake Onuma (Table 3)

a) *Hypomesus sakhalinus*, Ishikari-wakasagi (Plate I, Figs. b, c and d. Plate II, Figs. a and c. Plate III, Figs. b and c. Table 3, samples 16, 17a, 17c, 18a and 19a)

Hamada, K. (1957) reported this as a new species from Lake Taraika, southern Sakhalin. The pond smelt of Lake Ishikari-Furukawa was regarded as *H. olidus* until now. However, this school must be identified to *H. sakhalinus*, for they differ from *H. olidus* but agree entirely with the descriptions of *H. sakhalinus* reported by Hamada, K. (1957) in the taxonomic characteristics. Plate I, Figs. b and c show male and female of *H. sakhalinus* obtained from

[1] *Hypomesus sakhalinus* Hamada is given newly the Japanese name, **Ishikari-wakasagi.**

Table 3. *H. olidus* and *H. sakhalinus* obtained from Lake Ishikari-Furukawa and *H. olidus* obtained from Lake Onuma

No. of Sample	Locality	Date	Species	Number of Individuals		Body Length (mm)
				Male	Female	
16	Lake Ishikari-Furukawa	Apr. 24, 1950	*H. sakhalinus*	100	8*	111—46.5
17-a	Lake Ishikari-Furukawa	Apr. 18, 1950	*H. sakhalinus*	52	23	82—47.9
17-b	Lake Ishikari-Furukawa	Apr. 18, 1950	*H. olidus*	8	23	95.6—56
17-c	Lake Ishikari-Furukawa**	Apr. 20, 1953	*H. sakhalinus*	—	1	84.7
18-a	Lake Ishikari-Furukawa	Oct. 11, 1956	*H. sakhalinus*	41	42	103.4—55.8
18-b	Lake Ishikari-Furukawa	Oct. 11, 1956	*H. olidus*	10	10	94—68.8
19-a	Lake Ishikari-Furukawa	Apr. 26, 1957	*H. sakhalinus*	40	59	116.6—53.2
19-b	Lake Ishikari-Furukawa	Apr. 26, 1957	*H. olidus*	0	3	112.9—91
20	Lake Onuma	Apr. 13, 1948	*H. olidus*	18	33	76—43

* One specimen is *H. olidus* which was confused with *H. sakhalinus*.

** This specimen was caught on April 20, 1953 and was reared in the artificial pond for 25 weeks and 3 days.

Lake Ishikari-Furukawa.

The taxonomic characteristic are as follows:

94 mm in body length, male, Lake Ishikari-Furukawa, Hokkaido, Japan, April 24, 1950. Depth 18.4%; head 22.3% of body. Maxillary 36.1%, with minute canine-like teeth; snout 25.3%; orbit 26.2%; interorbital width 28.4% of head length. Snout shorter than interorbital width, 90% of interorbital width. Depth of caudal peduncle 31.2% of head length. D. 10; A. 16; P. 12; V. 8, inserted a little before origin of dorsal; fins larger than those of *H. olidus*, the pectoral 75% of p-v; number of vertebrae 53; teeth canine-like, small in number, in nearly biserial arrangement on vormer and palatine bones; pyloric caeca small, 3 in number, two pyloric caeca on right side of stomach and one on left. Pneumatic duct inserted about 1/5 distance from tip of air-bladder; lateral line incomplete, external openings 7. Minute excrescences on head, body side and fins. Color like that of *H. olidus*.

Body length 78 mm, female, Lake Ishikari-Furukawa, Hokkaido, Japan, April 24, 1950. Depth 19.8%; head 24.6% of body length. Maxillary 37.4%, with minute canine-like teeth; snout 24.8%; orbit 24.8%, interorbital width 26.4% of head length. Snout short, 94.1% of interorbital width. Depth of caudal peduncle 33.6% of head length. D. 9; A. 15; P. 12; V. 8, inserted a little before origin of dorsal; pectoral fin 61.4% of p-v; Lat. l. 55; vertebrae 55; teeth canine-like, small in number, in nearly biserial arrangement on vormer

and palatine bones; pyloric caeca small, 2 in number, being on each side of stomach. Pneumatic duct inserted about 1/5 distance from tip of air-bladder; lateral line incomplete, external openings 11. Color like *H. olidus*.

The characteristics observed on samples 16*, 17a, 17c, 18a and 19a are as follows:

D. 9 to 12; A. 14 to 19; P. 9 to 13; V. 8; vertebrae 50 to 57, with one exceptional specimen found in sample 19a (46 vertebrae), commonly 53 or 54; pyloric caeca small, 2 to 4 in number, almost always 2 or 3, rarely more, or less, and lacking; pneumatic duct inserted about 1/5 to 1/6 distance from tip of air-bladder; snout equal to interorbital width or shorter, almost always shorter; gill-rakers 28 to 34; head length 19 to 26% of body; diameter of orbit 18 to 27% of head, usually 20 to 24%; maxillary 29 to 40%. Minute excrescences on head, body side and fins of male fish during the breeding season.

b) *Hypomesus olidus* obtained from Lake Ishikari-Furukawa (Table 3, samples 17b, 18b and 19b)

These samples were caught together with *H. sakhalinus*.

D. 10 to 12; A. 16 to 19; P. 12 to 13; vertebrae 53 to 59; pyloric caeca 4 to 9; snout longer than interorbital width; head length 22 to 24% of body; diameter of orbit 18 to 24% of head; length of maxillary 37 to 47% of head.

c) *Hypomesus olidus* obtained from Lake Onuma (Table 3, sample 20)

H. olidus inhabiting Lake Onuma has no water-way to descend into the sea, that is to say they are a land-locked form. The characteristic are as follows:

D. 9 to 10; A. 14 to 17; P. 10 to 13; vertebrae 54 to 57; snout longer than interorbital width or equal; gill-rakers 31 to 36, commonly 33 or 34; length of maxillary 38 to 45% of head.

D) *Hypomesus olidus* obtained from many lakes in Honshu (Main Island of Japan) and Kyushu

The pond smelt inhabiting Lake Kogawara and Lake Kasumigaura have the possibility to descend into the sea and to ascend into the lake from the sea, though there is no certain report. R. Sato, H. Kato and T. Koji (1951) suggest that the pond smelt may ascend from the sea into Lake Kogawara. On the other hand, the pond smelt inhabiting the other lakes shown in table IV are land-locked forms and so it is not, entirely or almost, able to be believed that the pond smelt of these lakes ascend from the sea into the lake. The pond smelt obtained from Lake Kogawara and Lake Kasumigaura are separated therefor, from the pond smelt of the other lakes.

a) *Hypomesus olidus* obtained from Lake Kogawara (Table 4, sample 21).
Body length 49.6 to 107 mm

---

* One specimen of *H. olidus* was confused with sample 16; body length 125 mm. female, D. 11; A. 16; P. 13; vertebrae 56 (Plate II, Fig. b.).

Table 4. *H. olidus* obtained from lakes in the Main Island and Kyushu, Japan

No. of Sample	Locality	Date	Species	Number of Individuals		Body Length (mm)
				Male	Female	
21	Lake Kogawara	Mar. 24, 1959	*H. olidus*	95	5	49.5—107
22	Lake Suwa	May 17, 1956	*H. olidus*	24	28	59.5—79.3
23	Lake Yunoko	April 1951	*H. olidus*	12	11	65.6—118.3
24	Lake Kasumiga-ura (Aso)	Oct. 25, 1958	*H. olidus*	44	31	63.4—94.6
25	Lake Kasumiga-ura (Kihara)	Dec. 10, 1958	*H. olidus*	17	21	42.8—92
26	Lake Kawaguchi	Mar. 5, 1959	*H. olidus*	4	8	87.4—110
27	Lake Yamanaka	Apr. 6, 1959	*H. olidus*	43	44	63.2—82.9
28	Lake Kizaki	Apr. 8, 1959	*H. olidus*	27	73	71—103
29	Lake Ikeda	May 23, 1958	*H. olidus*	20	33	46.2—71.8

D. 9 to 11; A. 16 to 20; P. 12 to 14; vertebrae 54 to 58 in number, mostly 55 or 56; gill-rakers 8 to 11+19 to 25; pyloric caeca 3 to 6 in number; snout longer than interorbital width; head 23 to 27% of body length; diameter of orbit 21 to 27% of head; maxillary 39 to 45% of head.

b) *Hypomesus olidus* obtained from Lake Kasumigaura (Table 4, samples 24 and 25)

b_1) *Hypomesus olidus* obtained from the Aso water basin which is near the outlet of lake (sample 24)

Body length 63.4 to 94.6 mm.

D. 9 to 11; A. 15 to 19; P. 11 to 14; vertebrae 54 to 58 in number; gill-rakers 8 to 7+21 to 25; pyloric caeca 3 to 7 in number; snout longer than interorbital width; head 22 to 25% of body length; diameter of orbit 20 to 26% of head; maxillary 39 to 43% of head.

b_2) *Hypomesus olidus* collected from the Kihara which is the western region of Lake Kasumigaura (sample 25)

Body length 42.8 to 92 mm.

D. 9 to 11, 17 rays in one specimen; A. 15 to 18, 10 rays in one specimen; P. 11 to 14; vertebrae 53 to 58 in number; gill-rakers 8 to 10+20 to 26; pyloric caeca 3 to 7 in number; snout longer than interorbital width; head 23 to 26% of body length; diameter of orbit 21 to 29% of head; maxillary 39 to 44% of head.

c) *Hypomesus olidus* obtained from Lakes Suwa, Yunoko, Kawaguchi, Yamanaka, Kizaki and Ikeda (Table 4, samples 22, 23, 26 to 29)

Body length 46.2 to 118.3 mm.

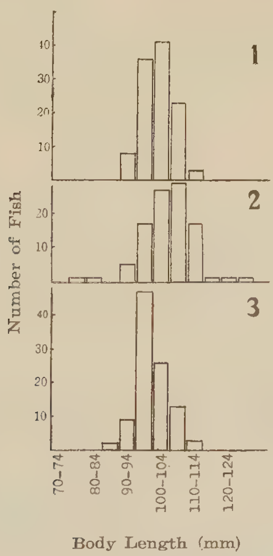
D. 9 to 12; A. 14 to 19; P. 10 to 15; vertebrae 54 to 58, 52 in one specimen; gill-rakers 8 to 11+20 to 26; pyloric caeca 3 to 7 in number; snout longer than interorbital width; head 20 to 27% of body length; diameter of orbit 18 to 30% of head; maxillary 36 to 44% of head, 33% in one specimen.

E) *Hypomesus japonicus*

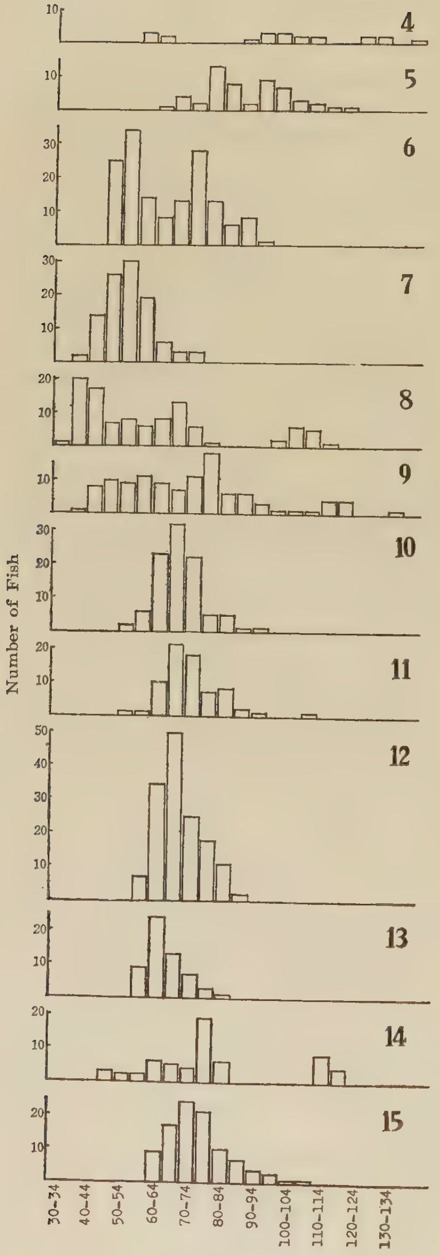
H. japonicus has not been found in fresh waters. All samples used in the present study were obtained from the coast of Hokkaido.

Table 5. *H. japonicus* obtained from the coast of Mori, Hakodate, Muroran and Lake On-neto

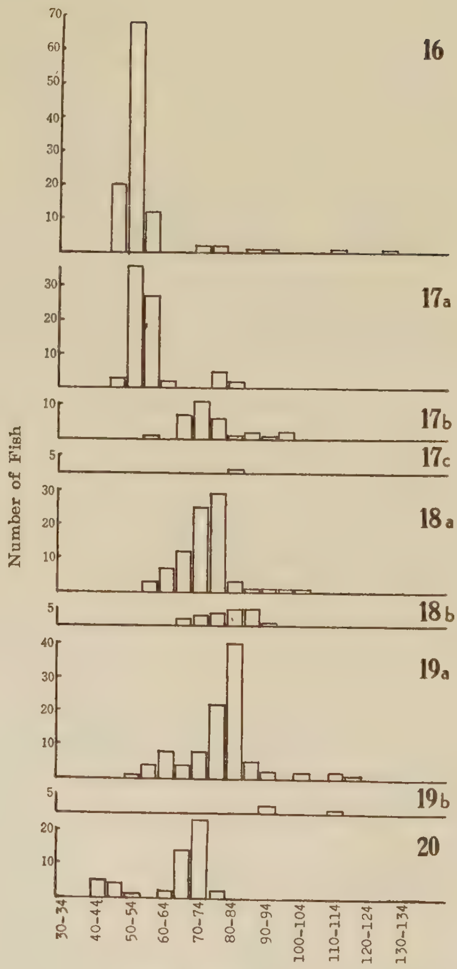
No. of Sample	Locality	Date	Species	Number of Individuals		Body Length (mm)
				Male	Female	
30	Coast of Mori	Sept. 14, 1954	*H. japonicus*	105		67—40.3
31	Coast of Mori	Oct. 25, 1954	*H. japonicus*	23	24	77.6—58
32	Coast of Mori	Nov. 18, 1954	*H. japonicus*	39	26	95—74.3
33	Coast of Mori	Nov. 19, 1954	*H. japonicus*	18	10	95—76.8
34	Coast of Mori	Nov. 27, 1954	*H. japonicus*	33	21	97.9—78.5
35	Coast of Mori	Apr. 20, 1955	*H. japonicus*	39	20	151.8—72.7
36	Coast of Mori	June 27, 1955	*H. japonicus*	10	15	123.6—100.4
37	Coast of Mori	May 1, 1957	*H. japonicus*	21	74	155.8—104.2
38	Coast of Hakodate	Apr. 10, 1954	*H. japonicus*	64	17	132.4—101.8
39	Coast of Muroran	Nov. 16, 1952	*H. japonicus*	26	29	107.1—79.5
40	Lake On-neto	Dec. ?, 1953	*H. japonicus*	29	21	175.6—98
41	Lake On-neto	Feb. 10, 1956	*H. japonicus*	14	8	100.5—77



Text-fig. 1. Body length frequencies of *H. olidus* obtained from the river Ishikari. 1: *H. olidus* obtained on May 27, 1949 (Table 1, sample 1). 2: *H. olidus* obtained on May 25, 1956 (Table 1, sample 2). 3: *H. olidus* obtained on May 19, 1957 (Table 1, sample 3).



Text-fig. 2.



Text-fig. 3.

a) *Hypomesus japonicus* obtained from the coast of Mori (Table 5, samples 30 to 37)

Body length 40.3 to 155.3 mm.

D. 9 to 13; A. 12 to 17; P. 11 to 15; vertebrae 60 to 66, mostly 64; pyloric caeca 4 to 8; snout shorter than interorbital width commonly, sometimes longer especially in young; length of maxillary 30 to 39% of head, commonly 33 to 36%.

b) *Hypomesus japonicus* obtained from the coast of Hakodate (Table 5, sample 38, Plate III, Fig. d)

Body length 101.8 to 132.4 mm.

D. 10 to 13; A. 13 to 18; P. 12 to 15; vertebrae 61 to 65, usually 63; pyloric caeca 4 to 7; snout shorter than interorbital width; length of maxillary 33 to 37% of head.

c) *Hypomesus japonicus* obtained from the coast of Muroran (Table 5, sample 39)

Body length 79.5 to 107.1 mm.

D. 10 to 12; A. 14 to 17; P. 12 to 15; vertebrae 62 to 66; pyloric caeca 4 to 7; snout almost always equal to diameter of orbit; length of maxillary 32 to 37% of head.

d) *Hypomesus japonicus* obtained from Lake On-neto, a salt lake (Table 5, samples 40 and 41)

Body length 77 to 175.6 mm.

D. 9 to 12; A. 13 to 17; P. 11 to 16; vertebrae 62 to 67; pyloric caeca 4 to 7; snout equal to interorbital width; length of maxillary 34 to 39% of head.

IV. Taxonomic Review

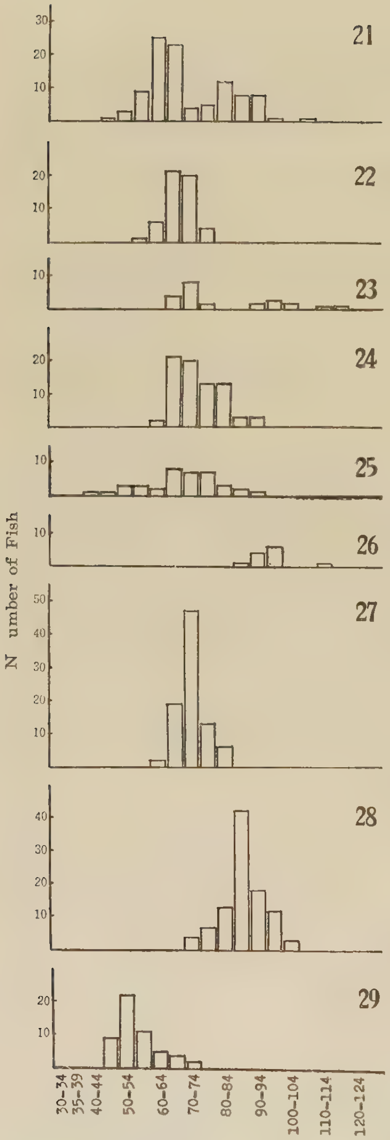
1) Review of scientific names

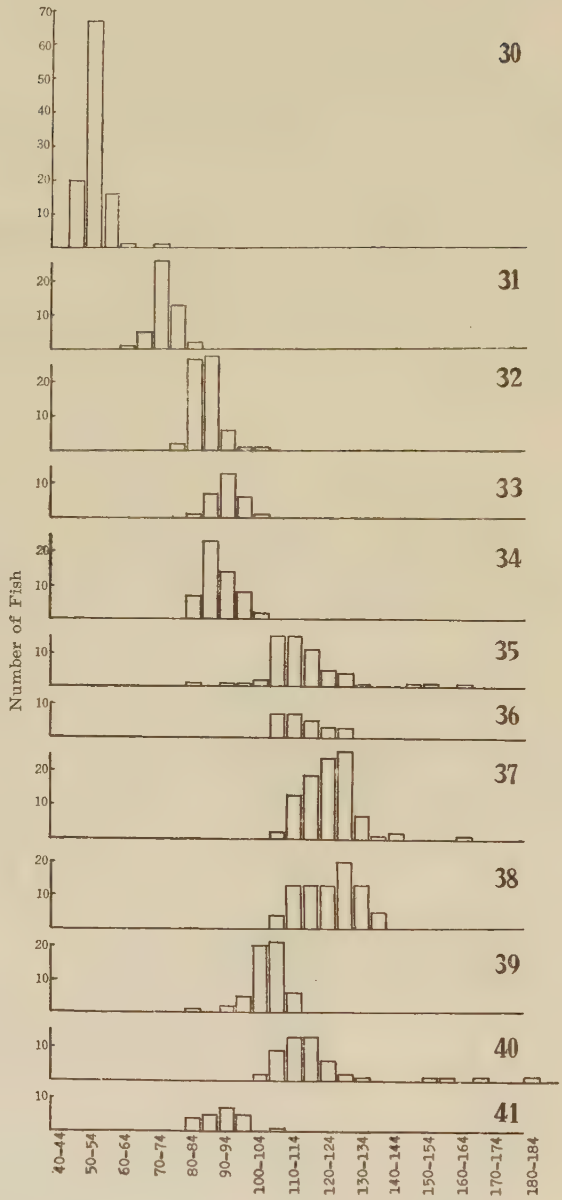
The species, belonging to Gen. *Hypomesus*, which found in the world are as follows:

Hypomesus olidus (PALLAS)
Hypomesus olidus bergi TARANETZ

Text-fig. 2. Body legnth frequencies of *H. olidus*. Samples 4 to 9 are specimens obtained from Lake Abashiri. Samples 10 and 11 are specimens obtained from the river emptying Lake Abashiri. Sample 12 is the specimens obtained from the Port of Abashiri. Sample 13 is obtained from the coast of Abashiri. Sample 14 comprises specimens obtained from Lake Tofutsu. Sample 15 is obtained from the coast of Abuta. The collection dates of these samples are shown in table 2.

Text-fig. 3. Body length frequencies of *H. olidus* and *H. sakhalinus*. Samples 16, 17a, 17c, 18a and 19a are *H. sakhalinus* obtained from Lake Ishikari-Furukawa. Samples 17b, 18b and 19b are *H. olidus* obtained from Lake Ishikari-Furukawa. Sample 20 is *H. olidus* obtained from Lake Onuma. The collection dates of these samples are shown in table 3. In sample 16, the largest specimen in body length is *H. olidus* which was confused with *H. sakhalinus*.





Text-fig. 4. Body length frequencies of *H. olidus*. Samples 21 to 28 were obtained from lakes in the Main Island, Japan, and sample 29 from Lake Ikeda, Kyushu. The data of the samples are shown in table 4.

Text-fig. 5. Body length frequencies of *H. japonicus*. Samples 30 to 37 are *H. japonicus* obtained from the coast of Mori. Sample 38 is specimens obtained from the coast of Hakodate. Sample 39 is *H. japonicous* obtained from the coast of Muroran. Samples 40 and 41 are *H. japonicus* obtained from Lake On-neto. The collection dates of these samples are shown in table 5.

Table 6. Frequencies of the number of dorsal rays observed in samples 1 to 20

No. of Sample	Number of Dorsal Rays 9	10	11	12	13	14	15
1	1	98	4				
2	1	28	69	2			
3		22	74	4			
4	2	19					
5	13	39	1				
6	56	88	6				
7–9			———				
10	33	60	3				
11	10	19	1				
12	47	99	3				
13	13	38					
14			———				
15	12	67	26	2			1
16	33	65	9*	1			
17–a–c			———				
18–a	2	31	47	3			
18–b			16	4			
19–a	9	52	34	4			
19–b		1	1	1			
20	25	26					

* One specimen having 11 rays is *H. olidus*.

Table 7. Frequencies of the number of dorsal rays observed in samples 21 to 29

No. of Sample	Number of Dorsal Rays 9	10	11	12	····	17
21	9	60	31			
22	25	27				
23	5	16	2			
24	5	59	11			
25	4	22	11			1
26	5	7				
27	34	49	4			
28	14	67	19			
29	3	38	11	1		

Table 8. Frequencies of the number of dorsal rays observed in samples 30 to 41

No. of Sample	Number of Dorsal Rays 9	10	11	12	13
30					
31		15	30	2	
32			———		
33			———		
34	1	15	30	8	
35		10	34	15	
36		6	15	3	
37		4	48	42	1
38		7	50	23	1
39		11	34	10	
40		6	34	10	
41	1	10	9	2	

Hypomesus olidus drjagini TARANETZ
Hypomesus sakhalinus HAMADA
Hypomesus vercundus JORDAN & METZ
Hypomesus japonicus (BREVOORT)
Hypomesus pretiosus (GIRARD)

Of these species and subspecies, *H. olidus*, *H. sakhalinus* and *H. japonicus* are recorded from Japan. *H. japonicus* was identified with *H. olidus* by Soldatov, V. K. & Lindberg, G. L. (1930) and Berg, L. S. (1932). Schmidt, P. U. (1904) stated that *H. pretiosus* may be a local form of *H. olidus*. That is to say,

Table 9. Frequencies of the number of anal rays observed in samples 1 to 20

No. of Sample	Number of Anal Rays								
	12	13	14	15	16	17	18	19	20
1				6	57	43	5		
2					9	44	42	4	1
3					10	52	32	5	1
4			3	6	9	2	1		
5			1	7	25	19	1		
6			1	2	37	89	21		
7–9			—	—	—	—	—		
10				5	43	34	13	1	
11	1	0	1	3	13	11	1		
12				4	52	67	24	2	
13			2	9	22	17	1		
14			—	—	—	—	—		
15			8	15	55	30			
16			6	54	38	10*			
17–a–c			—	—	—	—	—		
18–a				28	47	5	1	2	
18–b					1	13	4	2	
19–a			6	35	37	18	3		
19–b					1	1	1		
20			2	15	24	10			

* One specimen having 17 rays is *H. olidus*.

Table 10. Frequencies of the number of anal rays observed in samples 21 to 29

No. of Sample	Number of Anal Rays							
	10	14	15	16	17	18	19	20
21				16	54	23	6	1
22			5	26	18	3		
23				5	12	6		
24			12	32	26	4	1	
25	1		3	21	6	7		
26					5	4	3	
27			2	14	39	29	3	
28		1		9	49	32	9	
29				5	24	21	3	

Table 11. Frequencies of the number of anal rays observed in samples 30 to 41

No. of Sample	Number of Anal Rays						
	12	13	14	15	16	17	18
30			—	—	—	—	
31			8	20	13	6	
32, 33			—	—	—	—	
34		1	10	18	19	6	
35			4	21	22	12	
36	2	0	3	13	5	2	
37			7	34	47	7	
38		2	3	21	42	12	1
39				2	27	22	4
40				3	20	23	4
41			1	3	8	9	1

he regarded *H. pretiosus* as a synonym of *H. olidus*. Taranetz, A. (1936) asserted his opinion that *H. japonicus* is a synonym of *H. pretiosus*, and that *H. olidus* and *H. pretiosus* are independent from each other. He indicated also that the figure shown in Berg's paper is evidently *H. pretiosus*. Moreover, he added that segregation of distribution of one species is rare. Andriyashev, A. P. (1954) followed Taranetz's opinion stated above, and considered *H. japonicus* obtained from the Far East as a subspecies of *H. pretiosus*. Schmidt, P. U. (1950) followed after Taranetz's opinion, namely he separated *H. pretiosus* from *H. olidus*, and regarded *H. pretiosus* and *H. japonicus* as the same species. Then, he described that the specimens collected as recognizably different from

H. pretiosus inhabiting the coast of America by some characters. Jordan, D. S. & Gilbert, C. H. (1883) distinguished the independence of *H. pretiosus* from *H. olidus*. According to them, there are 70 scales on the lateral line of *H. pretiosus* and 56 to 60 scales on that of *H. olidus*. However, *H. japonicus* which had been collected at Petropaulovsk by them was erroneously identified with *H. olidus* as was later pointed out by C. L. Hubbs. They compared the specimens collected at Petropaulovsk and Eturup Island, and stated: "We do not venture to separate the two lots on the basis of our limited material, ———. It seems probable that we are dealing either with one species or with three or four."

Evermann, B. W. & Goldsborough, E. L. (1906), Günther, A. (1866), Hubbs, C. L. (1925) and Jordan, D. S. & Evermann, B. W. (1896) reported

Table 12. Frequencies of the number of pectoral rays observed in samples 1 to 20

No. of Sample	Number of Pectoral Rays						
	9	10	11	12	13	14	15
1				21	84	6	
2				23	73	4	
3				14	78	8	
4			3	12	5	1	
5			2	38	13		
6				71	78	1	
7–9			—	—	—		
10				59	35	1	1
11			1	12	15	2	
12				64	81	4	
13				11	39	1	
14			—	—	—		
15			—	—	—		
16	1	2	37	64	4*		
17–a–c			—	—	—		
18–a		4	34	42	3		
18–b				2	18		
19–a		2	37	56	4		
19–b				1	2		
20		3	5	36	7		

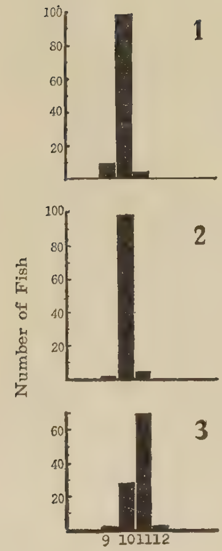
* One specimen having 13 rays is *H. olidus*.

Table 13. Frequencies of the number of pectoral rays observed in samples 21 to 29

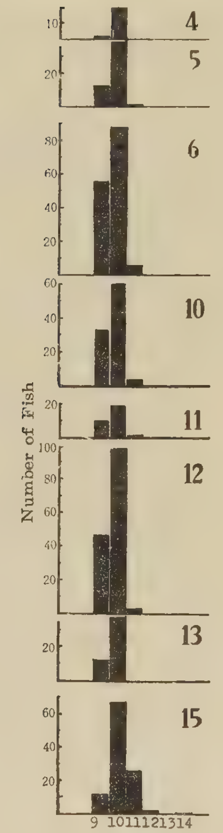
No. of Sample	Number of Pectoral Rays					
	10	11	12	13	14	15
21			42	50	8	
22		10	32	10		
23			6	16	1	
24		2	35	36	2	
25		2	20	15	1	
26		1	5	6		
27		4	46	36	1	
28	1	10	66	23		
29			6	32	14	1

Table 14. Frequencies of the number of pectoral rays observed in samples 30 to 41

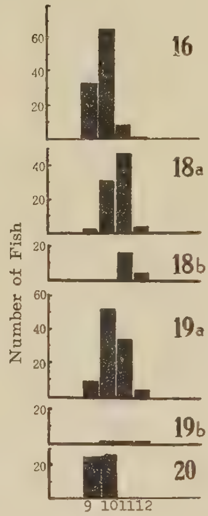
No. of Sample	Number of Pectoral Rays					
	11	12	13	14	15	16
30		—	—	—	—	
31	1	0	18	24	4	
32, 33		—	—	—	—	
34		2	24	25	3	
35		3	36	18	2	
36		2	9	14		
37		2	72	21		
38		10	41	28	2	
39		2	33	17	3	
40	1	0	8	34	6	1
41			9	11	2	



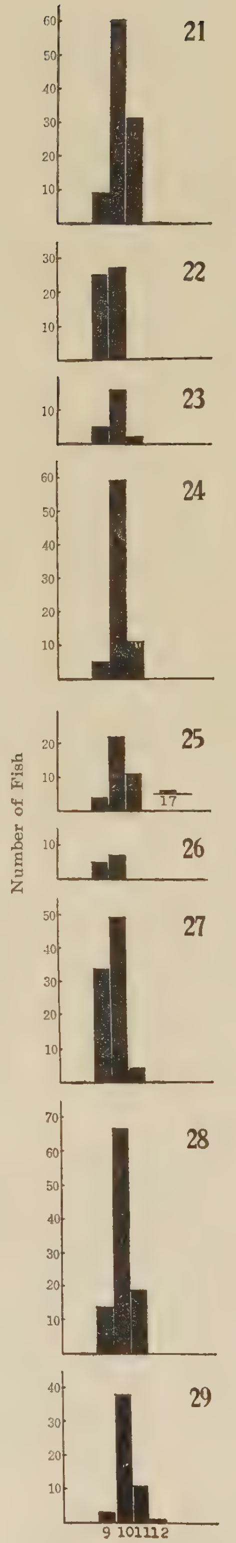
Number of Dorsal Rays
Text-fig. 6.



Number of Dorsal Rays
Text-fig. 7.



Number of Dorsal Rays
Text-fig. 8.



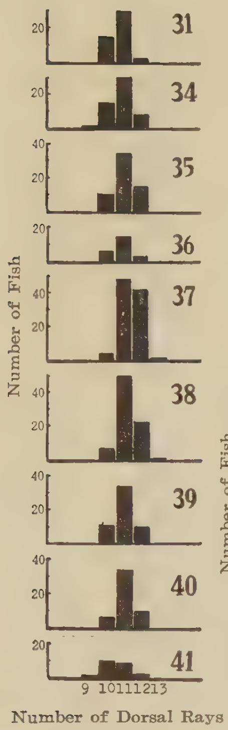
Number of Dorsal Rays
Text-fig. 9.

Text-fig. 6. Frequencies of the number of the dorsal rays of *H. olidus* obtained from the Ishikari River. The colletion dates are shown in table 1.

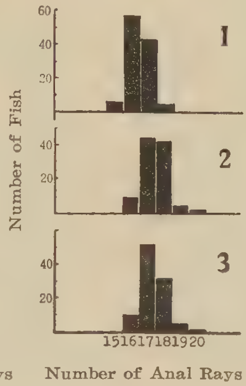
Text-fig. 7. Frequencies of the number of dorsal rays of *H. olidus*. Samples 4 to 6 are the specimens obtained from Lake Abashiri. Samples 10 and 11 are specimens obtained from a river flowing into Lake Abashiri. Sample 12 is specimens obtained from the Port of Abashiri. Sample 13 is specimens obtained from the coast of Abashiri. Sample 15 is specimens obtained from the coast of Abuta. The collection dates are shown in table 2.

Text-fig. 8. Frequencies of the number of the dorsal rays of *H. olidus* and *H. sakhalinus*. Samples 16, 18 a and 19 a are *H. sakhalinus* obtained from Lake Ishikari-Furukawa. Samples 18 b and 19 b are *H. olidus* obtained from Lake Ishikari-Furukawa. Sample 20 is specimens obtained from Lake Onuma. The collection dates of these samples are shown in table 3. In sample 16, one specimen having 11 rays is *H. olidus*.

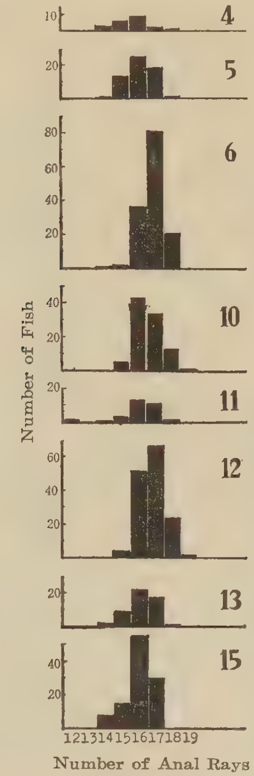
Text-fig. 9. Frequencies of the number of dorsal rays of *H. olidus*. Sample 21 to 28 are fish obtained from lakes in the Main Island and sample 29 is specimens obtained from Lake Ikeda, Kyushu. The data to the samples are shown in table 4.



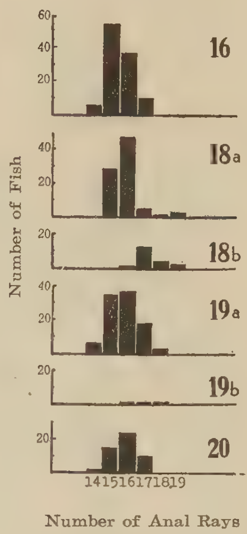
Text-fig. 10.



Text-fig. 11.



Text-fig. 12.



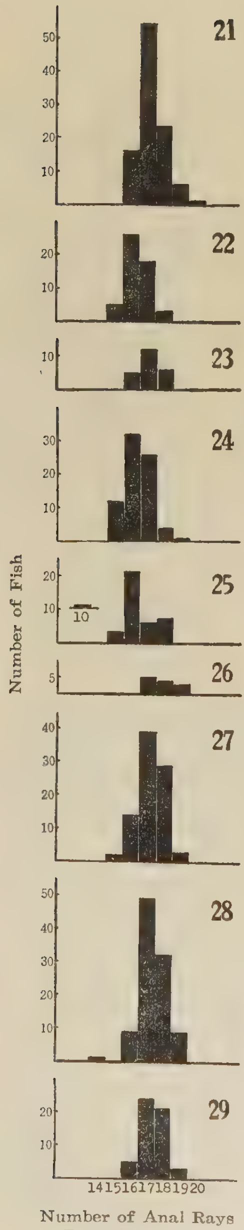
Text-fig. 13.

Text-fig. 10. Frequencies of the number of dorsal rays of *H. japonicus*. Samples 31 and 34 to 37 are specimens obtained from the coast of Mori. Sample 38 is specimens obtained from the coast of Hakodate. Sample 39 is specimens obtained from the coast of Muroran. Samples 40 and 41 are specimens obtained from Lake On-neto. The collection dates of these samples are shown in table 5.

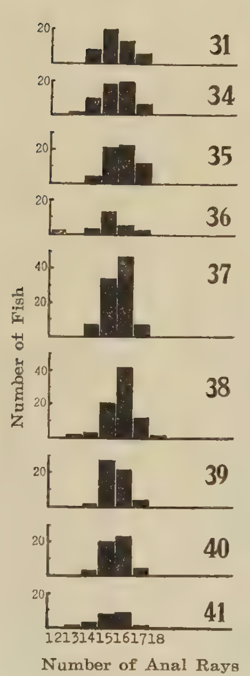
Text-fig. 11. Frequencies of the number of anal rays of *H. olidus* obtained from the river Ishikari. The collection dates of these specimens are shown in table 1.

Text-fig. 12. Frequencies of the number of anal rays of *H. olidus*. Samples 4, 5 and 6 are specimens obtained from Lake Abashiri. Samples 10 and 11 are specimens obtained from a river flowing into Lake Abashiri. Sample 12 is specimens obtained from the Port of Abashiri. Sample 13 is specimens obtained from the coast of Abashiri. Sample 15 is specimens obtained from the coast of Abuta. The collection dates are shown in table 2.

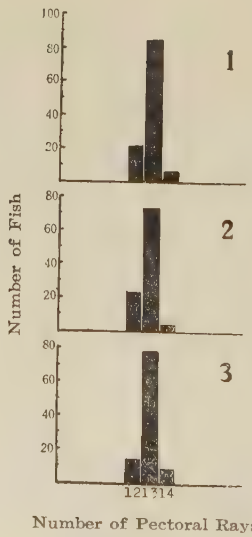
Text-fig. 13. Frequencies of the number of anal rays of *H. olidus* and *H. sakhalinus* obtained from Lake Ishikari-Furukawa. Samples 16, 18a and 19a are *H. sakhalinus* obtained from Lake Ishikari-Furukawa. Samples 18b and 19b are *H. olidus* obtained from the same lake. Sample 20 is *H. olidus* obtained from Lake Onuma. The collection dates are shown in table 3. In sample 16, one specimen having 16 rays is *H. olidus*.



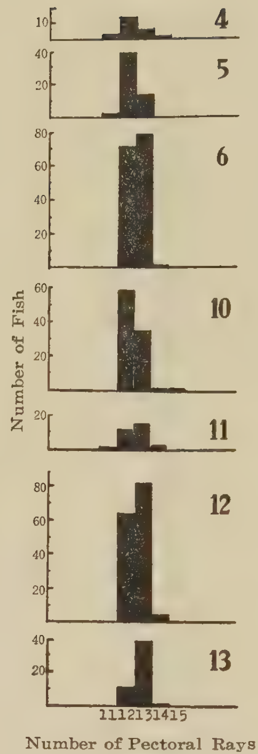
Text-fig. 14.



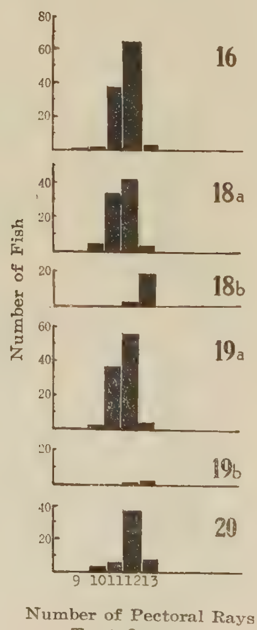
Text-fig. 15.



Text-fig. 16.



Text-fig. 17.



Text-fig. 18.

H. olidus and *H. japonicus*. Jordan, D. S. and Metz, C. W. (1913) found a species, *Hypomesus vercundus*, from the waters of Korea. However, according to Hamada, K. (1954), *H. vercundus* may be a synonym of *H. japonicus*. He recently reported a species, *H. sakhalinus* from Lake Taraika, southern Sakhalin (1957). And two subspecies, *H. olidus bergi* and *H. olidus drjagini* were found by Taranetz, A .(1937) from northern Sakhalin. *H. sakhalinus* was distinguished by some difference of characters, especially the form of the air-bladder, from other species belonging to Gen. *Hypomesus*. *H. olidus bergi* was separated from *H. olidus*, by such characters as the larger eye, the small number of pyloric caeca and the small size of the body. *H. olidus drjagini* was separated from *H. olidus* by the number of pyloric caeca, being always two.

2) Review of *H. olidus*, *H. sakhalinus* and *H. japonicus*

H. olidus is found commonly along the coast of North Japan, in the Okhotsk Sea, the Bering Sea, and the Amur River (P. U. Schmidt 1950, A. P. Andriyashev 1954). In Japan, *H. olidus* was artificially transplanted into many lakes; the species breeds extensively in various districts at present. Such a wide distribution gives to expectation that *H. olidus* may show many local forms. If there is a remarkable modification of the form within *H. olidus*, this modification may lead to confusion for the classification of Gen. *Hypomesus*. It is based on the variability of the vertebral number that some authors regard *H. japonicus* as a synonym of *H. olidus*. The writer compared the morphological characters of *H. olidus* with those of *H. sakhalinus* and *H. japonicus* to make clear the differences among these three species.

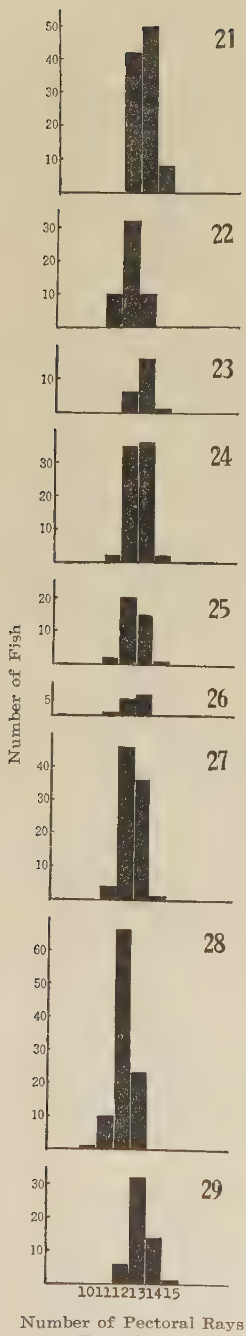
Text-fig. 14. Frequencies of the number of anal rays of *H. olidus*. Samples 21 to 28 are specimens obtained from certain lakes in the Main Island and sample 29 is specimens obtained from Lake Ikeda, Kyushu. The data of samples are shown in table 4.

Text-fig. 15. Frequencies of the number of anal rays of *H. japonicus*. Samples 31 and 34 to 37 are specimens obtained from the coast of Mori. Sample 38 is obtained from the coast of Hakodate. Sample 39 is specimens obtained from the coast of Muroran. Sample 40 and 41 are *H. japonicus* obtained from Lake On-neto. The collection dates are shown in table 5.

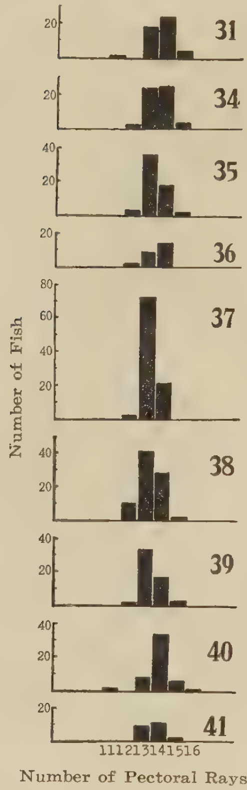
Text-fig. 16. Frequencies of the number of pectoral rays of *H. olidus* obtained from the river Ishikari. The collection dates of these specimens are shown in table 1.

Text-fig. 17. Frequencies of the number of pectoral rays of *H. olidus*. Samples 4, 5 and 6 are specimens obtained from Lake Abashiri. Samples 10 and 11 are the specimens obtained from a river emptying into Lake Abashiri. Sample 12 is specimens obtained from Port Abashiri, and sample 13 from the coast of Abashiri. The collection dates are shown in table 2.

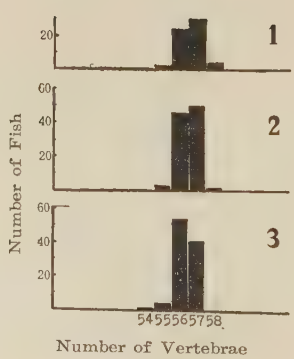
Text-fig. 18. Frequencies of the number of pectoral rays of *H. olidus* and *H. sakhalinus*. Samples 16, 18a and 19a are *H. sakhalinus* obtained from Lake Ishikari-Furukawa. Samples 18b and 19b are *H. olidus* from Lake Ishikari-Furukawa. Sample 20 is *H. olidus* obtained from Lake Onuma. The collection dates are shown in table 3. In sapmle 16, one specimen having 13 rays is *H. olidus*.



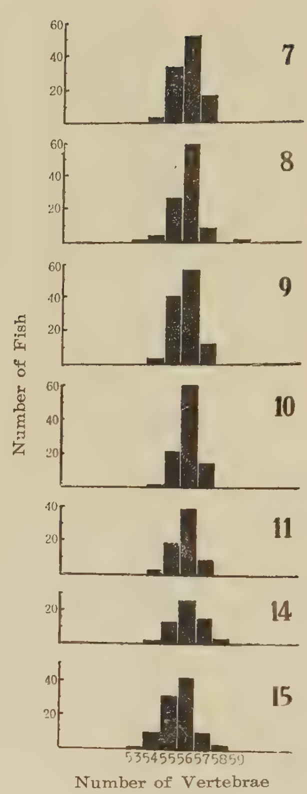
Text-fig. 19.



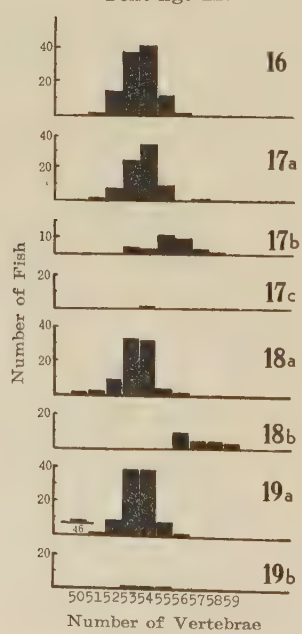
Text-fig. 20.



Text-fig. 21.



Text-fig. 22.



Text-fig. 23.

a) Number of fin rays (Tables 6 to 14, Text-figs. 6 to 20)

In Gen. *Hypomesus,* there is no considerable difference in the number of dorsal rays and anal rays (Text-figs. 6 to 15) among species. The number of pectoral rays of *H. olidus* obtained from Lake Onuma is evidently small as compared with the others (Text-fig. 18, sample 20), namely the mode of the frequency of the number of pectoral rays is 12. In the pond smelts of the Main Island, there is also a tendency toward a decrease of the number of rays (samples 22, 25, 27 and 28). On the other hand, the pectoral rays in *H. sakhalinus* are fewer in number, the writer counts only 11 or 12 rays in the greater part of them (Text-fig. 18, samples 16, 18a and 19a). *H. japonicus* has more numerous pectoral rays than *H. olidus* and *H. sakhalinus* as is indicated in Text-fig. 20 (13 or 14 rays almost always). This fact that *H. japonicus* has more numerous rays may be remarked in relation with the ecology. *H. olidus* obtained from Lake Abashiri and adjacent waters is intermediate in number of the pectoral rays between the two specimens obtained from the river Ishikari and Lake Onuma (Text-fig. 17). All species belonging to Gen. *Hypomesus* have 8 ventral rays with extremely rare exception.

b) Number of vertebrae (Tables 15, 16, 17, Text-figs. 21 to 25)

In Gen. *Hypomesus,* difference in number of vertebrae is evidently admitted between *H. japonicus* and the other species. The vertebral number of *H. japonicus* is greater than in the others except *H. pretiosus,* and the vertebrae of *H. olidus* are few as compared with other species except *H. sakhalinus.* It is also recognized that there is a racial difference within *H. olidus.* In the specimens obtained from the Ishikari River, the number of vertebrae is mostly

Text-fig. 19. Frequencies of number of pectoral rays of *H. olidus.* Samples 21 to 28 are the fish obtained from lakes in the Main Island and sample 29 is specimens obtained from Lake Ikeda, Kyushu. The data of samples are shown in table 4.

Text-fig. 20. Frequencies of number of pectoral rays of *H. japonicus.* Samples 31 and 34 to 37 are specimens obtained from the coast of Mori. Sample 38 is specimens obtained from the coast of Hakodate. Sample 39 is specimens obtained from the coast of Muroran. Samples 40 and 41 are specimens obtained from Lake Onneto. The collection dates of these samples are shown in table 5.

Text-fig. 21. Frequencies of the number of vertebrae of *H. olidus* obtained from the river Ishikari. The collection dates of these samples are shown in table 1.

Text-fig. 22. Frequencies of the number of vertebrae of *H. olidus.* Samples 7, 8 and 9 are specimens obtained from Lake Abashiri. Samples 10 and 11 are specimens obtained from a river flowing into Lake Abashiri. Sample 14 is specimens obtained from Lake Tofutsu, and sample 15 from the coast of Abuta. The collection dates of these samples are shown in table 2.

Text-fig. 23. Frequencies of the number of vertebrae of *H. olidus* and *H. sakhalinus.* Samples 16, 17a, 17c, 18a and 19a are *H. sakhalinus* obtained from Lake Ishikari-Furukawa. Samples 17b, 18b and 19b are *H. olidus* from Lake Ishikari-Furukawa. The collection dates are shown in table 3. In sample 16, one of two specimens having 56 vertebrae is *H. olidus.*

Table 15. Frequencies of the number of vertebrae observed in samples 1 to 20

No. of Sample	Number of Vertebrae									
	50	51	52	53	54	55	56	57	58	59
1						2	24	30	4	
2						3	46	50	1	
3					1	4	54	41		
4–6	—									
7					3	33	51	16		
8				2	5	26	58	9		1
9					3	40	56	12		
10					1	21	60	14		
11					3	19	39	9		
12, 13	—									
14					2	13	26	15	3	
15				1	10	31	42	10	3	
16		1	14	37	42	12	2*			
17–a		1	7	24	33	9				
17–b				4	3	11	9	3	1	
17–c					1					
18–a	1	2	9	33	32	4	1			
18–b	(46)						9	4	4	3
19–a	1	1	9	39	39	8	2			
19–b				1	1	1				
20	—									

* One specimen having 56 vertebrae is *H. olidus*.

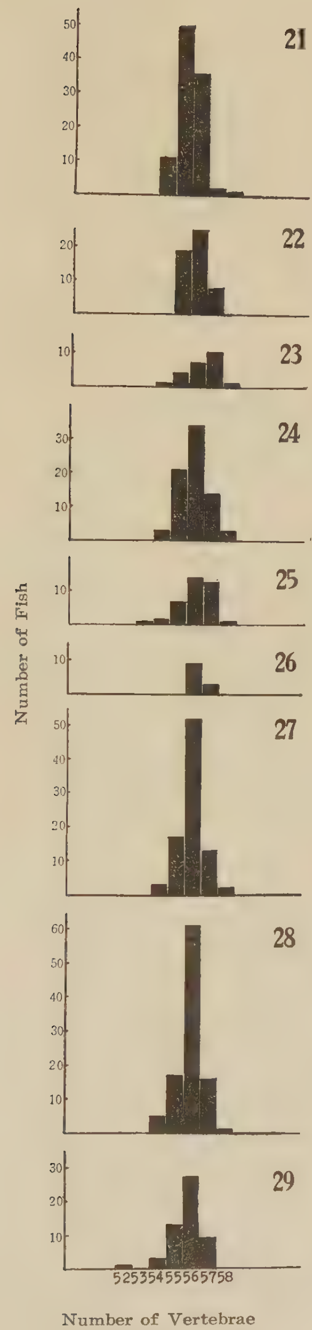
Table 16. Frequencies of the number of vertebrae observed in samples 21 to 29

No. of Sample	Number of Vertebrae						
	52	53	54	55	56	57	58
21			11	50	36	2	1
22				19	25	8	
23			1	4	7	10	1
24			3	21	34	14	3
25		1	2	7	14	13	1
26					9	3	
27			3	17	52	13	2
28			5	17	61	16	1
29	1		3	13	27	9	

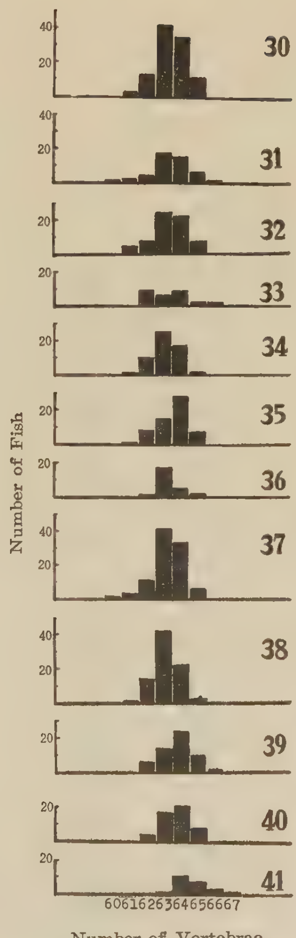
Table 17. Frequencies of the number of vertebrae observed in samples 30 to 41

No. of Sample	Number of Vertebrae							
	60	61	62	63	64	65	66	67
30		3	13	42	35	11		
31	1	2	4	17	15	6	1	
32		4	7	24	22	7		
33			9	6	9	2	2	
34		1	10	25	17	1		
35		1	8	15	28	7		
36			1	17	5	2		
37	1	3	11	41	33	6		
38		1	14	42	22	2		
39			6	14	24	10	1	
40			4	17	21	8		
41				1	10	7	3	1

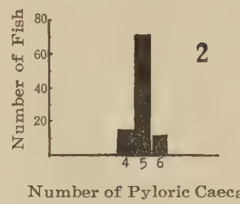
56 or 57 (Text-fig. 21). The vertebral number of *H. olidus* obtained from Lake Abashiri and the adjacent waters is less than that of the specimens collected from the Ishikari River. Their vertebrae are mostly 55 to 56 in number (Text-fig. 22). The specimens collected from Lake Tofutsu, the coast of Abuta, Lake Abashiri, lakes of the Main Island and Kyushu are similar in number of vertebrae (Text-figs. 22, 24). On *H. olidus* inhabiting Lake Ishikari-Furukawa, there are some doubts as to the validity of inferences from the writer's samples, for the specimens are extremely few in number as shown in table 3. However, it may be inferred that the number of vertebrae of these specimens is similar to



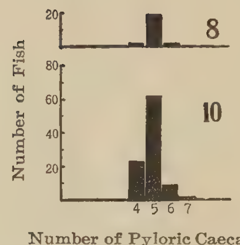
Text-fig. 24.



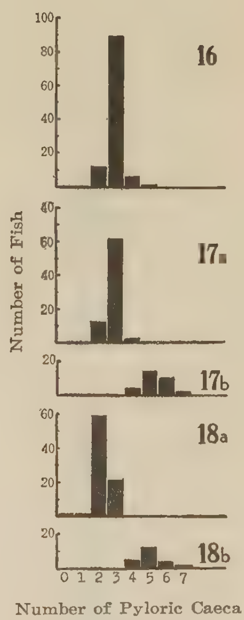
Text-fig. 25.



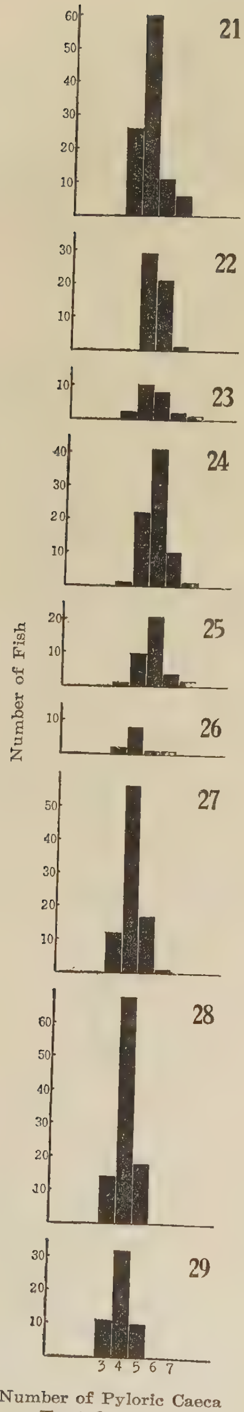
Text-fig. 26.



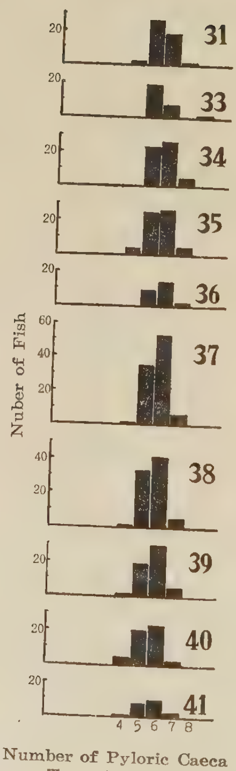
Text-fig. 27.



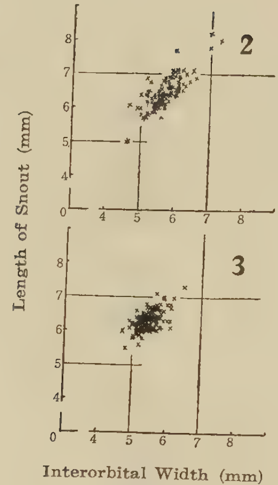
Text-fig. 28.



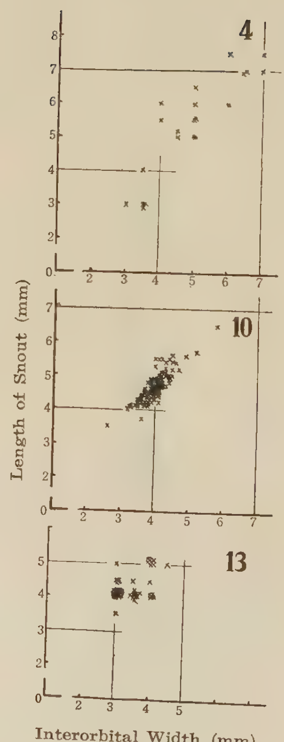
Text-fig. 29.



Text-fig. 30.



Text-fig. 31.



Text-fig. 32.

that of the specimens collected from other waters (Text-fig. 23, 17b, 18b and 19b). The vertebral number of *H. olidus* inhabiting Lake Onuma was not counted. According to R. Sato (1951), such number ranges from 54 to 57, with the mode of frequency at 56. On the other hand, it is rare that *H. sakhalinus* has 56 vertebrae, commonly furnishing with 52 to 55, and mostly 53 or 54 (Text-fig. 23, samples 16, 17a, 17c, 18a and 19a). The vertebrae of *H. japonicus* range from 60 to 67 in number being mostly 63 to 64 (Text-fig. 25). Then *H. japonicus* is distinguished in respect to vertebral number from *H. olidus* and *H. sakhalinus*.

As stated above, there is a difference in vertebral number between *H. olidus* and *H. sakhalinus*. And *H. japonicus* shows the numerous number of vertebrae which is the most remarkable character for separation of this species from the others.

c) Number of pyloric caeca (Tables 18, 19 and 20, Text-figs. 26 to 30)

Text-figure 26 shows the frequency of the number of pyloric caeca of *Hypomesus olidus* obtained from the Ishikari River (sample 2). Their pyloric caeca range from 4 to 6 in number, but mostly 5. The specimens collected from Lake Abashiri and a flowing river are similar to the specimens from the river Ishikari in number of pyloric caeca (Text-fig. 27). Also the number of pyloric caeca does not distinguish *H. olidus* collected in Lake Ishikari-Furukawa from *H. olidus* inhabiting the other waters (Text-fig. 28, samples 17b and 18b). *H. olidus* inhabiting lakes of the Main Island shows 3 to 7 pyloric caeca, with the mode of frequency 4, excepting the samples collected from Lake Kasumigaura (Text-fig. 29). The mode of frequency of the specimens obtained from that lake is 5 (samples 24 and 25). *H. sakhalinus* has almost always 2 or 3 pyloric caeca (Text-fig. 28, samples 16, 17a and 18a). That is to say, the pyloric caeca of *H. sakhalinus* are less than those of *H. olidus* in number.

Text-fig. 24. Frequencies of the number of vertebrae of *H. olidus*. Samples 21 to 28 are specimens obtained from certain lakes in the Main Island and sample 29 is specimens obtained from Lake Ikeda, Kyushu. The date of samples are shown in table 4.

Text-fig. 25. Frequencies of the number of vertebrae of *H. japonicus*. Samples 30 to 37 are specimens obtained from the coast of Mori. Sample 38 is specimens obtained from the coast of Hakodate. Sample 39 is specimens obtained from the coast of Muroran. Samples 40 and 41 are specimens obtained from Lake On-neto. The collection dates of these samples are shown in table 5.

Text-fig. 26. Frequencies of the number of pyloric caeca of *H. olidus* obtained in the river Ishikari on May 25, 1956.

Text-fig. 27. Frequencies of the number of pyloric caeca of *H. olidus*. Sample 8 is specimens obtained from Lake Abashiri on Sept. 29, 1953, and sample 10 from a river flowing into Lake Abashiri on April 26, 1955.

Text-fig. 28. Frequencies of the number of pyloric caeca of *H. olidus* and *H. sakhalinus*. Samples 16, 17a and 18a are *H. sakhalinus* and samples 17b and 18b are *H. olidus* obtained from Lake Ishikari-Furukawa. The collection dates are shwon in table 3. In sample 16, one specimen having 56 vertebrae is *H. olidus*.

According to Taranetz, A. (1936, 1937), and Schmidt, P. U. (1950), *H. olidus bergi* and *H. olidus drjagini* are separated from *H. olidus* by the few number of pyloric caeca.

H. japonicus has 4 to 8 pyloric caeca, the most part of the specimens having 5 or 6 (Text-fig. 30). Therefore, there is no clear difference between *H. olidus* and *H. japonicus*. The pyloric caeca of *H. japonicus* are however remarkably longer than those of *H. olidus* as was pointed out by Hamada, K. (1954).

d) Length of the snout relative to the interorbital width (Text-figs. 31 to 35)

The length of the snout of *H. olidus* obtained from the Ishikari River is always greater than the interorbital width as is shown in Text-fig. 31. In *H. olidus* from Lake Abashiri and adjacent waters, the length of snout is also greater than the interorbital width with a few exceptions in which the two dimensions are equal; especially, in the specimens of small size the snout is rather shorter than the interorbital width (Text-fig. 32). In *H. olidus* obtained from Lake Ishikari-Furukawa and Lake Onuma, the relative length of the snout to interorbital width is also similar to that of *H. olidus* collected in Lake Abashiri. Excepting a few extremely tiny fish, there is no specimen which has the snout shorter than the interorbital width (Text-fig. 33, samples 18b, 19b and 20). In this relation, the specimens collected from lakes of the Main Island and Kyushu are not dissimilar to *H. olidus* inhabiting Lake Ishikari-Furukawa and Lake Onuma (Text-fig. 33). Contrariwise, the length of the snout of *H. sakhalinus* is shorter than the interorbital width, with a few rare exceptions (Text-fig. 33, samples 18a and 19a). This is one of the remarkable characteristics distinguishing *H. sakhalinus* from *H. olidus*. In some samples of *H. japonicus*, the snout is equal to the interorbital width on the average (Text-fig. 35, samples 31, 39 and 40), however in others, the snout is shorter than the interorbital width for the most part of the specimens (Text-fig. 35, samples 34 and 35). Further, in certain other samples, the

Text-fig. 29. Frequencies of the number of pyloric caeca of *H. olidus*. Samples 21 to 28 are specimens obtained from lakes in the Main Island. Sample 29 is specimens obtained from Lake Ikeda, Kyushu. The data of samples are shown in table 4.

Text-fig. 30. Frequencies of the number of pyloric caeca of *H. japonicus*. Samples 31 and 33 to 37 are specimens obtained from the coast of Mori. Sample 38 is the specimens obtained from the coast of Hakodate and sample 39 from the coast of Muroran. Samples 40 and 41 are specimens obtained from Lake On-neto. The collection dates are shown in table 5.

Text-fig. 31. Length of the snout relative to the interorbital width in *H. olidus* obtained from the river Ishikari. Sample 2 was collected on May 25, 1956, and sample 3 on May 19, 1957.

Text-fig. 32. Length of the snout relative to the interorbital width in *H. olidus*. Sample 4 is specimens obtained from Lake Abashiri on Oct. 23, 1947. Sample 10 is specimens obtained on April 26, 1955 from a river emptying into Lake Abashiri. Sample 13 is a specimens obtained from the coast of Abashiri on Sept. 21, 1950.

Table 18. Frequencies of the number of pyloric caeca observed in samples 1 to 20

No. of Sample	Number of Pyloric Caeca							
	0	1	2	3	4	5	6	7
1			—					
2					15	71	12	
3–7			—					
8					2	19	2	
9			—					
10					23	62	9	1
11–15			—					
16			12	89	6	1*		
17–a			12	61	2			
17–b					4	14	10	2
17–c			1					
18–a	1	1	59	22				
18–b					4	12	3	1
19–20			—					

* This specimen is *H. olidus*.

Table 19. Frequencies of the number of pyloric caeca observed in samples 21 to 29

No. of Sample	Number of Pyloric Caeca					
	3	4	5	6	7	?
21	26	60	11	1		2
22		29	21	1		1
23	2	10	8	2	1	
24	1	22	41	10	1	
25	1	10	21	4	1	1*
26	2	8	1	1		
27	12	56	17	1		1**
28	14	68	18			
29	11	32	10			

Table 20. Frequencies of the number of pyloric caeca observed in samples 30 to 41

No. of Sample	Number of Pyloric Caeca				
	4	5	6	7	8
30			—		
31	4	26	18	1	
32			—		
33		19	7	0	1
34		23	26	5	
35	4	25	26	4	
36		9	14	2	
37	1	35	53	6	
38	1	33	41	5	
39	1	19	30	5	
40	4	20	23	2	
41	1	8	10	3	

snout is shorter than the interorbital width with a few rare exception (Text-fig. 35, samples 37 and 38). The snout of some specimens of *H. japonicus* is longer than the interorbital width, but it is shorter in the others. This fact may be related with the sexual maturity of the fish.

As stated above, *H. sakhalinus* is distinguished, commonly, from *H. olidus* by the snout which is shorter than the interorbital width.

e) Number of gill-rakers (Tables 21 and 22)

The number of gill-rakers has not been given the exhaustive attention as

Table 21. Frequencies of the number of gill-rakers observed in samples 1, 4, 5, 6, 12, 13, 16 and 20

		Number of Sample							
		1	4	5	6	12	13	16	20
Number of Gill-Rakers: Upper Lobe	8		1						
	9	4	9	1	0	3	1	3	1
	10	97	10	43	17	73	28	13	47
	11	10	1	9	115	66	19	55	3
	12				18	7	3	13	
Lower Lobe	18							4	
	19		1	0	0	0	0	19	
	20		1	0	0	0	0	28	
	21	1	1	0	5	4	0	27	
	22	11	6	0	41	14	2	5	2
	23	42	8	6	79	78	11	1	28
	24	53	4	21	23	46	24	0	16
	25	3	0	24	2	6	13	0	5
	26	1	0	2	0	1	1		
Total	28		1	0	0	0	0	2	
	29		1	0	0	0	0	8	
	30							17	
	31	3	1	0	4	2	1	25	1
	32	11	2	0	12	9	1	20	1
	33	35	5	5	33	47	7	11	26
	34	54	10	18	72	60	17	1	18
	35	7	1	25	16	25	16	0	4
	36			5	13	5	7	0	1
	37	1	0	0	0	1	2		

the taxonomic characteristic until now. In the present study, the writer observed the taxonomic significance of this character on some samples of *H. olidus* and *H. sakhalinus*. In the former, the mode of frequency of the number of gill-rakers is 32 to 35, being in most samples 33 or 34, as shown in text-figures 36 and 37, and tables 21 and 22, while in *H. sakhalinus* it is 31. The vertebrae and the pyloric caeca of *H. sakhalinus* are fewer than those of *H. olidus* in number as already stated, and the gill-rakers are also less in number.

f) Length of the head relative to the length of body (Tables 23 and 24)

Hamada, K. (1954) reported that no clear difference in length of the head relative to that of the body between *H. olidus* and *H. japonicus* is recognizable. Then, in this study, the writer does not carry out the comparison of the two. Tables 23 and 24 show the percentage of the head length to the body length of

Table 22. Frequencies of the number of gill-rakers observed in samples 21 to 28

			Numple of Sample								
			21	22	23	24	25	26	27	28	29
Number of Gill-Rakers	Upper Lobe	8	2	3	2	2	3	2	3	8	2
		9	38	25	21	33	17	3	44	61	17
		10	58	23		33	17	7	40	31	33
		11	2	1		7					1
		?					1				
	Lower Lobe	19	1								
		20	3	1			1				
		21	2	7	7	1	1		2	2	1
		22	24	11	5	7	5		6	31	8
		23	45	25	11	35	20		31	38	25
		24	22	8		27	5	9	39	27	12
		25	3			5	4	3	8	2	7
		26					1		1		
		?					1				
	Total	29	1	1	2		1				
		30	5	6	5		2		3	6	2
		31	12	11	5	8	4		4	26	3
		32	34	14	11	20	10	2	19	24	15
		33	28	13		23	13	4	33	33	16
		34	17	7		18	4	4	23	10	12
		35	2			5	2	2	4	1	5
		36	1			1	1		1		
		?					1				

H. olidus and *H. sakhalinus*. According to these tables it seems that there is no difference between the two species. The percentage varies widely however with the different waters.

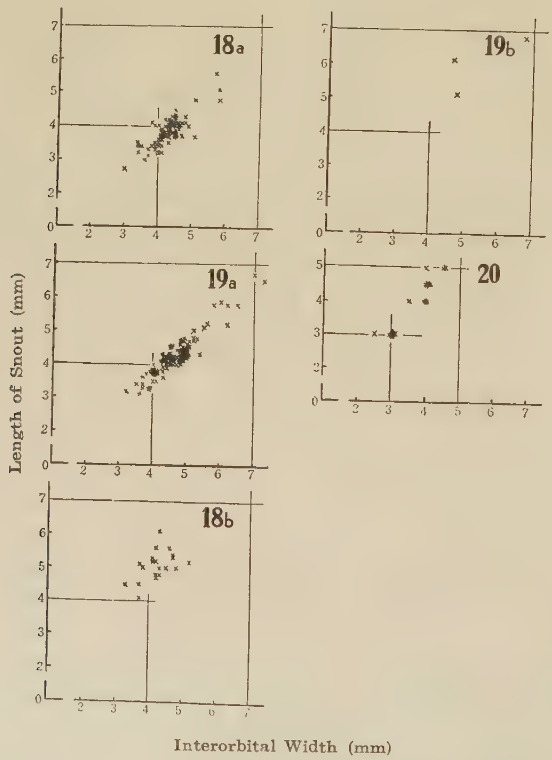
g) Length of the diameter of orbit relative to the head length (Table 25 and 26)

There is no remarkable difference between *H. olidus* and *H. sakhalinus* in the length of the diameter of orbit relative to length of the head. The diameter of the orbit is 18 to 30% of the head length in *H. olidus*, and 18 to 27% in *H. sakhalinus*.

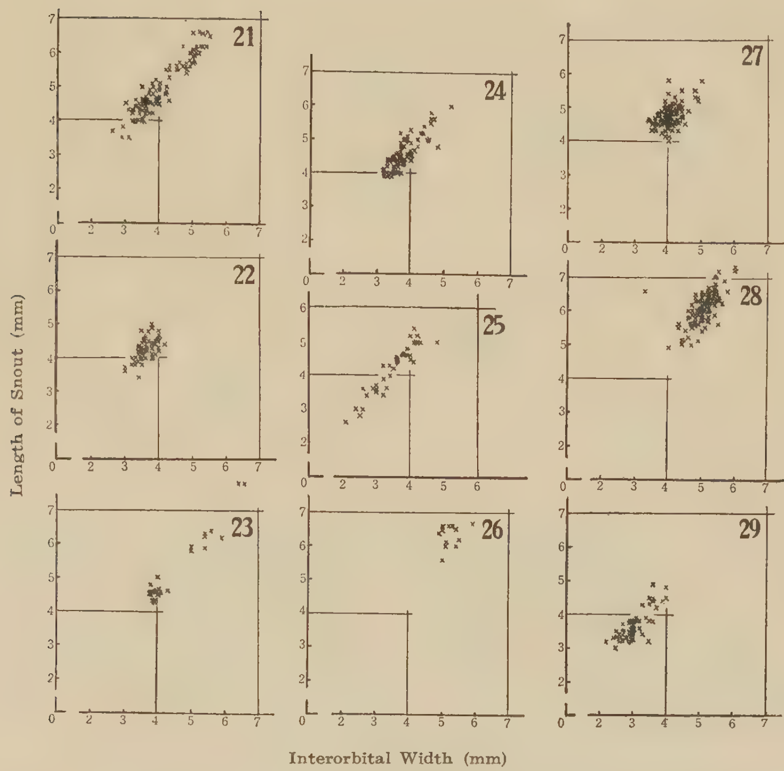
h) Length of the maxillary relative to the head length (Tables 27, 28 and 29)

According to Taranetz, A. (1936, 1937), the maxillary of *H. pretiosus* (=

H. japonicus) is shorter than that of *H. olidus* in length. Hamada, K. (1954) reported also that *H. olidus* was distinguished from *H. japonicus* by the larger length of the maxillary. Tables 27 and 28 show the relative length of the maxillary to the head in *H. olidus* (samples 2, 3, 4, 8, 10, 14, 18b, 19b, 20 and 21 to 29) and in *H. sakkalinus* (samples 18a and 19a). The maxillary of *H. olidus* is obviously longer than that of *H. sakhalinus*. Namely, the length of the maxillary of *H. olidus* shows 36 to 50% (largely 38% and over), with one exceptional specimen which showed 33%, of the length of head, while that of *H. sakhalinus* is only 29 to 40% (largely 35% and less) of the length of the head. *H. japonicus* is nearly the same, in the length of the maxillary relative to the head, as *H. sakhalinus* being less than 40% of the length of head (Table 29). Therefore, *H. olidus* is distinguished from *H. sakhalinus* and *H. japonicus* by the longer length of the maxillary.



Text-fig. 33. Length of the snout relativ to the interorbital width in *H. olidus* and *H. sakhalinus*. Samples 18 a and 19 a are *H. sakhalinus*, and samples 18b and 19b are *H. olidus* obtained from the river Ishikari-Furukawa. Sample 20 is *H. olidus* obtained from Lake Onuma. The collection dates are shown in table 3.

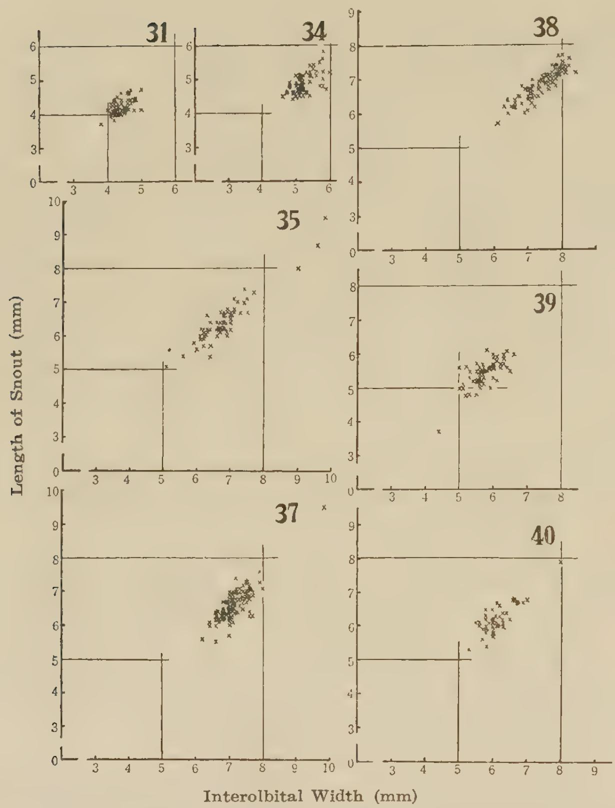


Text-fig. 34. Length of the snout relative to the interorbital width in *H. olidus*. Samples 21 to 28 are specimens obtained from lakes in the Main Island and sample 29 is specimens obtained from Lake Ikeda, Kyushu. The data of samples are shown in table 4.

3) Comparison of *Hypomesus pretiosus*, *H. vercundus*, *H. olidus bergi* and *H. olidus drjagini*

H. pretiosus differs sharply from *H. olidus* as has been pointed out by Hubbs, C. L., but there is no clear difference between *H. japonicus* and *H. pretiosus*. The latter is separated from *H. japonicus* by only the small size of the scales. Hubbs, C. L. (1925) enumerated 66 to 76 scales in the course of the lateral line of *H. pretiosus*, and Jordan, D. S. & Evermann, B. W. (1896) 70 scales. Excepting the scale size, Evermann, B. W. & Goldsborough, E. L. (1903) also did not recognize any difference between the two species. A. Taranetz's opinion that *H. japonicus* is a synonym of *H. pretiosus* might be caused by such indistinctness in difference of character. The writer has no good data upon which to come to a conclusion whether *H. pretiosus* and *H. japonicus* are different species or not. Therefore, he follows C. L. Hubbs's opinion, and distinguished *H. japonicus* from *H. pretiosus* for the time being.

According to Jordan, D. S. & Metz, C. W. (1913), *H. vercundus* has the characteristics as follows: D. 10; A. 13; V. 8; scales 60 to 64; depth 5.6; head



Text-fig. 35. Length of the snout relative to the interobital width in *H. japonicus*. Samples 31, 34, 35 and 37 are specimens obtained from the coast of Mori. Sample 38 is specimens obtained from the coast of Hakodate. Sample 39 is specimens obtained from the coast of Muroran, and sample 40 from Lake On-neto. The collection dates of these samples are shown in table 5.

Table 23. Percentage of the head length to the body length in samples 2, 3, 10, 18a, 18b, 19a and 19b

No. of Sample	Percentage of Head Length to Body Length											
	19	20	21	22	23	24	25	26	27	28	29	30
2			15	57	25	3						
3	1	4	10	54	30	1						
10				1	29	48	17	1	0	0	0	1
18–a		2	28	41	8	3	0	1				
18–b				3	9	8						
19–a	1	1	10	48	31	6	2					
19–b				1	1	1						

Table 24. Percentage of the head length to the body length in samples 21 to 29

No. of Sample	Percentage of Head Length to Body Length							
	20	21	22	23	24	25	26	27
21				4	26	51	7	2
22		4	20	27	1			
23	1	3	11	8				
24			2	24	39	10		
25				11	14	12	1	
26			1	3	4	2	0	2
27			3	25	45	11	3	
28			2	35	48	15		
29					16	31	4	2

Table 25. Percentage of the diameter of the orbit to the head length in samples 2, 3, 10, 18a, 18b, 19a and 19b

No. of Sample	Percentage of Diameter of orbit to Head Length											
	18	19	20	21	22	23	24	25	26	27	28	29
2		6	28	27	26	10	3					
3		2	9	36	32	14	5	0	1	1		
10	1	0	0	6	10	18	8	29	14	5	3	1
18-a	1	4	18	30	20	6	3	0	0	1		
18-b	1	0	3	9	3	2	2					
19-a		3	18	32	26	9	9	1	1			
19-b				2	1							

Table 26. Percentage of the diameter of the orbit to the head length in samples 21 to 29

No. of Sample	Percentage of Diameter of Orbit to Head Length												
	18	19	20	21	22	23	24	25	26	27	28	29	30
21				2	6	12	22	33	20	5			
22			2	6	18	17	6	3					
23		1	8	7	4	3							
24			2	6	15	25	16	10	1				
25				2	3	10	4	11	3	1	3	1	
26	1	0	2	1	3	4	1						
27			1	1	11	19	20	25	8	1	1		
28		2	2	12	27	24	15	12	3	3			
29						2	6	12	14	14	2	2	

5.5; eye 3.66 in head; snout 3.6; interorbital width 3.66; maxillary 2.5; number of branchiostegal rays 7; gill-rakers numerous, slender, about 12 to 24; ventral inserted under anterior third of dorsal. From this description, except that the ventral fins insert under anterior third of dorsal rays, we read the characteristics of *H. japonicus*. The position of the ventral fins may vary, to some extent, in accordance with the maturity or the growth. For this reason, in this case, it is doubtful whether the position of the ventral rays should be esteemed highly as a taxonomic characteristic. There is no evident difference between *H. japonicus* and *H. vercundus*. Assuming this to be the case, as the writer pointed out previously, it will be seen that *H. vercundus* is a synonym of *H. japonicus*.

According to Taranetz, A. (1937), *H. olidus bergi* is separated from *H. olidus olidus* by the small number of pyloric caeca (2 pyloric caeca), larger eye (32.1 to 36.3% of the head) and the small size of the body. This subspecies inhabits a small lake 6 km up the river Tuim from Nogulidi Village, northern Sakhalin. This lake does not connect with the river Tuim. That is to say,

Table 27. Percentage of the length of maxillary to the length of head in samples 2, 3, 4, 8, 10, 14, 18a, 18b, 19a, 19b and 20

Percentage of Length of Maxillary to Length of Head	Number of Sample: 2	3	4	8	10	14	18-a	18-b	19-a	19-b	20
29									1		
30									3		
31									4		
32							3		11		
33							11		16		
34							17		21		
35							26		23		
36							7		10		
37			1			1	13	1	6		
38	2		1			0	5	1	4	1	1
39	12	3	1		6	7	0	0		1	0
40	24	19	4	6	22	11	1	0		0	1
41	31	28	2	7	21	9		1		1	6
42	19	34	5	7	28	17		3			2
43	8	13	3	20	13	8		5			2
44	4	2	2	25	4	2		4			2
45			0	25	0	3		2			5
46			0	6	1	0		0			
47			0	5	0	1		3			
48			2		0						
49					0						
50					1						
51											

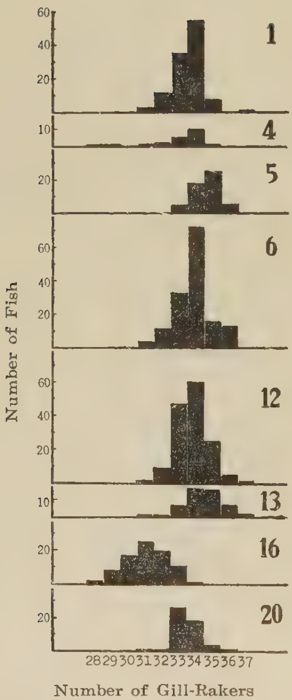
Table 28. Percentage of the length of the maxillary to the length of head in samples 21 to 29

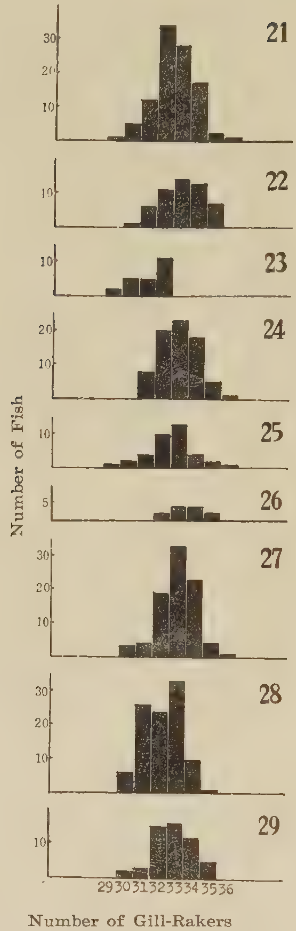
Percentage of Length of Maxillary to Length of Head	Number of Sample 21	22	23	24	25	26	27	28	29
33							1		
34							0		
35							0		
36		2			1	1	0		
37		5	4		0	5	0	4	
38		16	8		0	3	10	16	
39	1	15	5	6	4	2	32	27	2
40	5	7	3	13	6	1	24	38	12
41	17	7	3	21	10		17	13	16
42	37			24	11		3	2	13
43	28			11	2				7
44	8				4				3
45	4								

Table 29. Percentage of the length of the maxillary to the length of head in samples 35, 37, 38, 39 and 41

Percentage of Length of Maxillary to Length of Head	Number of Sample 35	37	38	39	41
30		1			
31		2			
32		4		1	
33	3	19	8	2	
34	16	36	18	14	3
35	20	23	38	15	5
36	12	9	14	13	6
37	5	1	3	10	6
38	2				1
39	1				1

H. olidus bergi is a land-locked form. *H. olidus drjagini* was found in a basin of the river Koluma, and was separated from *H. olidus olidus* on the basis of the small number of pyloric caeca (always two). According to Taranetz, A. (1937), the typical *H. olidus olidus* has three finger-like pyloric caeca. Hamada, K. (1954) reported that *H. olidus* has 4 to 7 pyloric caeca, Schmidt, P. U. (1950) described 3 or 4, and Andriyashev, A. P. (1954) noted 2 to 4 pyloric caeca. In the present study, 3 to 7 pyloric caeca are observed on the same species. The diameter of orbit of *H. olidus* is 30% and less of the head length. The larger



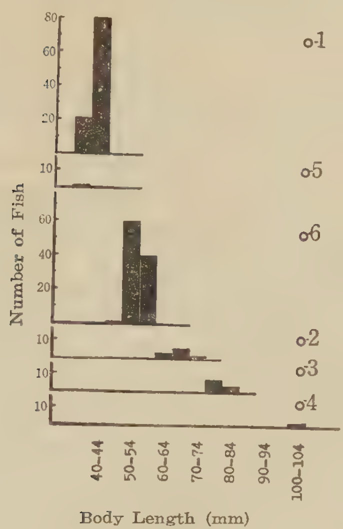


Text-fig. 36. Frequencies of the number of gill-rakers of *H. olidus* and *H. sakhalinus*. Sample 1 is *H. olidus* obtained from the river Ishikari. Samples 4, 5 and 6 are *H. olidus* obtained from Lake Abashiri. Sample 12 is *H. olidus* obtained from the Port of Abashiri, and sample 13 from the coast of Abashiri. Sample 16 is *H. sakhalinus* obtained from Lake Ishikari-Furukawa. Sample 20 is *H. olidus* obtained from Lake Onuma. The collection dates of these samples are shown in tables 1 to 3.

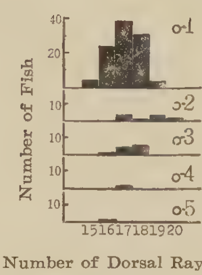
Text-fig. 37. Frequencies of the number of gill-rakers of *H. olidus*. Samples 21 to 28 are the specimens obtained from lakes in the Main Island and sample 29 is specimens obtained from Lake Ikeda, Kyushu. The data of samples are shown in table 4.

eye and the small size of the body in *H. olidus bergi* stand maybe in some relation to the special environment. *H. sakhalinus* has generally 2 or 3 pyloric caeca, and sometimes none (Hamada, K. 1957). Namely, *H. sakhalinus* is similar to *H. olidus bergi* and *H. olidus drjagini* in number of pyloric caeca. *H. sakhalinus*

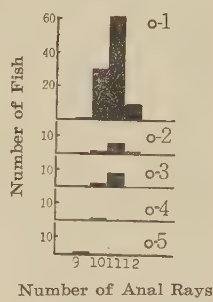
is however, separated from *H. olidus bergi* and *H. olidus drjagini* by the smaller orbit which is 27% or less of the head length, the snout which is shorter than the interorbital width and the difference of the connected position of the pneumatic duct with the air-bladder as stated before.



Text-fig. 0–I.



Text-fig. 0–II.



Text-fig. 0–III.

Text-fig. 0–I. Frequencies of the body length of *H. olidus* obtained from Oshoro Bay. The collection dates of these samples are shown in table 0–I.

Text-fig. 0–II. Frequencies of number of dorsal rays in *H. olidus* obtained from Oshoro Bay. The collection dates are shown in table 0–I.

Text-fig. 0–III. Frequencies of number of anal rays in *H. olidus* obtained from Oshoro Bay. The collection dates are shown in table 0–I.

V. Ecological Study

Taranetz, A. (1936), Schmidt, P. U. (1950) and Andriyashev, A. P. (1954) have still some doubts whether *H. japonicus* should be separated from *H. pretiosus* as a different species; some authors have also similar doubts on the relation between *H. olidus* and *H. japonicus*. Then the writer attempted to solve these questions through ecological study.

On the ecology of *H. olidus*, there are the papers by many authors such as Amemiya, I. & Hiyama, Y. (1940), Kobayashi, H. (1936), Sato, R. (1950) and Shiraishi, Y. (1952). Taranetz, A. (1936) referred to the ecology of *H. olidus* and *H. pretiosus* (=*H. japonicus*) in his paper on the taxonomy of the species. The writer carried on ecological studies on *H. olidus* inhabiting the lakes and the coast of Hokkaido and also on *H. japonicus* and *H. sakhalinus*.

The ecological study is important not only as a fundamental study for fishery biology but also in the relation with the taxonomy. It is found occa-

sionally to be difficult or nearly impossible to identify a species by only the morphological characters. A species has the characteristics of itself, and at the same time a species its own has peculiar ecological niche. The form of the living organisms was maybe formed through the entire history of its life, and it may be forming even now. Accordingly, the study of the ecology is not only important taxonomically, but is significant in the phylogenetical aspect.

1) *Hypomesus olidus*

H. olidus is separated by differences of habit into two forms and two types.

a) *a-type of anadromous form*

The writer applies the name "*a-type of anadromous form*" to *H. olidus* which ascends into the river to spawn in the spring. *H. olidus,* which ascends into the river Ishikari to spawn from the Sea of Japan in the middle of May or toward the end, belongs to this type (samples 1, 2 and 3). *H. olidus* belonging to this type is found in only spawning period in the river Ishikari. Their vertebrae are mostly 56 or 57 in number. Hamada, K. (1956) concluded that *H. olidus* found in Oshoro Bay is the young of the anadromous form ascending into the river Ishikari, through comparison of the number of vertebrae between *H. olidus* obtained from Oshoro Bay and *H. olidus* from the Ishikari River, and the investigations of the growth rate. According to Taranetz, A. (1936), the spawning school of *H. olidus* ascends into the river which flows into Peter the Great Bay, in the later part of April and early in May. Text-fig. 1 shows the frequency of the body length of specimens obtained from the river Ishikari. In Text-fig. 1, samples 1 and 3 are fish ascending to spawn into the river Ishikari, and sample 2 is a fish descending into the sea after spawning. In 1956, *H. olidus* ascended to spawn in the Ishikari River on about the 16th of May. The migration after spawning is not evident, but it is not an unwarrantable assumption that *H. olidus* which ascends in the river Ishikari closes his life after spawning, for in the next year, the ascending all *H. olidus* are one year old fish as was proved by Hamada, K. (1956). Sample 2 was obtained from near the mouth of the river, and the gonad was empty. Therefore, it is recognized that this school descends to the sea after spawning and dies in a short time. *H. olidus* belonging to the a-type of anadromous form and *Oncorhynchus masou* are exactly alike in their life history.

b) *b-type of anadromous form*

The writer applies the name "*b-type of anadromous form*" for *H. olidus,* which ascends into the fresh waters in the autumn and spawns after the close of the winter. Sano, S. (1937) reported that *H. olidus* inhabiting Lake Abashiri descends into the sea in the spring and ascends into the lake in the autumn. Hamada, K. (1953a, c) observed the same behavior; he says the fish descend into the sea in the spring, and ascend into the lake in the middle or last of October. The fish spawn in a flowing river or on the coast of the lake in the end of April. In this population, 0, 1 and 2 year old fish and rarely 3 and 4 year olds are found. Some of them do not descend to the sea, and their growth

is retarded in comparison with the fish which descend into the sea. Therefore, there are 1 age fish which are equal in size to 0 age fish, determined by the scale reading. According to the writer's observation hitherto, no essential difference is recognized in ecology and form between the descending fishes and undescending fishes. However, the some divergence in ecology is observable in the school of *H. olidus* inhabiting Lake Abashiri. According to Taranetz, A. (1936), a spawning school ascends in the river, which flows into Peter the Great Bay, in the end of April and the begining of May, and the other spawing school ascends in the same river at the begining of September. He stated that the latter school, ascending in the autumn, spawns evidently. Text-figure 2 shows the frequency of the body length of *H. olidus* obtained from Lake Abashiri, and some other waters (Table 2). Samples 4, 5, 6, 8 and 9 are from the schools which is composed of various age groups. But, it can not be assured that there are various age groups in samples 10, 11, 12 and 13. In sample 10, the annulus was found on the scales of six specimens. In sample 11, there were three specimens ornamented with the annulus on scales. Samples 10 and 11 are *H. olidus* which ascended to spawn in a river flowing into Lake Abashiri. These *H. olidus* on which are found annuli on the scales must be thought as 2 age fishes. Samples 12 and 13 are evidently from the schools which ascend into Lake Abashiri after a short time. Hamada, K. (1953c) presumed *H. olidus* which ascends into Lake Abashiri as 0 age fishes. This presumption is not erroneous in the light of the investigation on the growth rate of *H. olidus* found in Oshoro Bay. Text-figure o-I shows the growth rate of *H. olidus* found in that bay. In this figure, sample o-3 collected on Oct. 3, 1952 is 75 to 83 mm in body length. This length is just equal to the body length of *H. olidus* which ascends into Lake Abashiri in the autumn. *H. olidus* found in the bay of Oshoro in the autumn is 0 age fish. Accordingly, *H. olidus* which ascends into Lake Abashiri must be regarded as 0 age fish.

As stated above, *H. olidus* found in Lake Abashiri descends into the sea in the spring, and ascends to the lake in the autumn. Moreover, another school which lives in the lake through their whole life is found. This school is not a true land-locked form, but it includes residual fish of the descending school.

Sample 14 obtained from Lake Tofutsu on Sept. 29, 1953 may be separated into two groups; the main group is 84 mm and less in body length while the subgroup is 111 mm and over. The fish of the main group are covered with scales without annulus, while six specimens out of twelve specimens in the subgroup show one annulus and the others two annuli on their scales. Nevertheless, there is no difference in body length between the specimens with one annulus and those with two annuli. Such fact gives to one to doubt the age determination. However annuli on the scales show the age certainly. As stated above, some of *H. olidus* inhabiting Lake Tofutsu ascend into the lake from the sea in the autumn, and the others remain in the lake through their life. Interestingly, the growth rate of fish is so rapid in the sea that one age fish ascended from the sea are equal in body length to two age fish which remained in the lake. This fact is exceedingly similar to the phenomenon observed on *H. olidus* of Lake Abashiri. There were five specimens which have one annulus on their

scales and one specimen which has two annuli on his scales in 97 specimens obtained from the coast of Abuta on Nov. 23, 1952 (sample 15).

R. Sato (1950) observed the habit and age composition of the pond smelt in Lake Kogawara. He says "no racial difference exists within groups or between groups around Lake Kogawara, though the schools of the pond smelt ascend the Takase River toward Lake Kogawara before the spawning season (the end of March and early in April)." Although the writer also observed the meristic characters of *H. olidus* in Lake Kogawara, no racial difference was found (sample 21). However, there is a fish group showing no annulus on their scales in spite of the body length corresponding to that of the fish which show 1 annulus. Of specimens ranged within 70 to 94 mm in body length, 11 fish show no annulus on their scales, while twelve fish show 1 annulus (Text-fig. 4, sample 21). This fact demonstrates that 1 age fish which ascend into the lake from the sea are equal, in body length, to 2 age fish which reside in the lake. Lake Kogawara is similar to Lake Abashiri in ecological conditions for both lakes are connected with the sea by a river.

In Lake Kasumigaura, *H. olidus* spawns in the lake or the running stream, in January and February. There is no observation whether *H. olidus* ascends or not from the sea. The ascending fish is never found in the river flowing into the sea from the lake, and so, there is little doubt but that *H. olidus* inhabiting Lake Kasumigaura live in the lake throughout their life. Nevertheless it is considerable that the greater part of them have 5 pyloric caeca as against the fact that *H. olidus* inhabiting the other lakes of the Main Island has, have the most part, 4 pyloric caeca. The age composition was not examined for the reason of desquamation.

c) *land-locked form*

The land-locked form is the fish which can not descend to the sea on account of physical obstacles for example that the river is blocked up, as a result of the eruption of a volcano. Recently, Dr. Oshima, M. (1957) maintained his opinion, that it is not suitable strictly that *Oncorhynchus masou* inhabiting a river throughout its life is called "land-locked form", for the fish have the way to descend into the sea. The writer agrees entirely with Dr. Oshima's opinion.

For *H. olidus* inhabiting Lake Onuma there is not a way into the sea. That is, they are the fish of the land-locked form. The some of them die after spawn, and the others spawn again in the next year. The age of them is exactly determined by the body length, for the frequency distribution of their body length is clearly separated into two groups (Text-fig. 3, sample 20). The age is also determinable by scale reading (Hamada, K. 1953b). In Lake Onuma, *H. olidus* spawns at the beginning or the middle of April. *H. olidus* inhabiting Lake Shironuma may be the same life history with the fish of Lake Onuma, for the scale structures of fishes of both lakes resemble each other (Kobayashi, H. 1936, Hamada, K. 1953b).

Among the samples obtained from lakes in the Main Island and Kyushu, samples 22, 23, 26, 27, 28 and 29 are land-locked. All specimens collected from

Lake Suwa (sample 22), Lake Yamanaka (sample 27) and Lake Kizaki (sample 28) are one age fish (hatched in the preceding year, 1958), for the specimens have been collected in the close of the spawning season. Two age fish are found amongst the specimens collected from Lake Yunoko (sample 23), Lake Kawaguchi (sample 26) and Lake Ikeda (sample 29), and three age fish in the specimens from Lake Yunoko and Lake Kawaguchi. No remarkable difference is found among these land-locked fish as to the life-history which is nearly similar to that of the fish inhabiting Lake Onuma (sample 20). However, there is one interesting fact that the spawning period of *H. olidus* becomes longer according as it is found at lower latitudes. For example, the spawning period is about 7 days in Lake Onuma, 2 weeks in Lake Kogawara, 2 months in Lake Kasumigaura and 3 months or over in Lake Kizaki.

2) *Hypomesus sakhalinus*

H. sakhalinus lives in the fresh water (Lake Ishikari-Furukawa) throughout their life, and thence this species is not found in the sea. For maturity they take one years and spawn in the middle or end of April. The egg which is like that of *H. olidus* is commonly agglutinating on the tiny roots of willows which extend in the water of the lake. The embryo hatches after about 20 to 30 days at 7.5° to 9.5°C.

Text-figure 3 (samples 16, 17a, 17c, 18a and 19a) shows the length frequencies of the body of *H. sakhalinus*. Sample 16 is clearly composed of the different age fish. In particular, the fish of the main group are 49 mm and less in body length whilst the fish of the subgroup are 74 to 94 mm. One annulus is read on the scales of fish of the subgroup, but no annulus on the scales of the main group. The fishes of main group are 1 age and those of the subgroup are 2 age. One specimen which is 111 mm in body length shows 3 annuli on the scales, so it is a 4 age fish (Plate II, figs. a and c, Plate IV, fig. a). In sample 16, one specimen which is 125 mm in body length is *H. olidus* (Plate II, figs. b and d).

Sample 17a may be divided into two groups, the main group of fishes which is 64 mm and less in body length, and subgroup which is 75 mm and over. These groups may be considered as age groups. However, in the fishes of the subgroup which are larger in body length, an annulus is found only on the scales of three specimens among seven. This fact rakes doubt whether the annulus shows exactly the age of all fish or just some certain specific ones. In the paper on the jack smelt, *Atherinopsis californiensis*, F. N. Clark (1929) reported that no annulus was formed on the scales of 30 per cent of all his specimens. The annulus may also be not always formed on the scales in *H. sakhalinus*. Sample 17c is a specimen obtained from Lake Ishikari-Furukawa on April 20, 1953 and reared in the artificial pond until Oct. 15, 1953. The circuli of the scale showed irregular form in the region of the margin. Sample 18a is not divided into two age groups. The annulus is found on the scales of only one specimen which is 98 mm in body length. Sample 19 is composed of three age groups.

As stated above, the scale is not an adequate criterion for determination of the age of *H. sakhalinus*. The mechanism of the formation of the annulus is not certainly known at present, though retardation of growth in the winter

or in the spawning period is considered as the cause of the formation of an annulus.

The meristic characters displayed by an individual fish are determined not alone by heredity, but in part also by the environmental conditions particularly temperature, which prevail during some sensitive developmental period (Hubbs 1922). A fish has generally fewer vertebrae and a less protracted development in a comparatively higher temperature. A fish develops more slowly, and has more vertebrae in a lower temperature. The writer observed the development of *H. olidus* and *H. sakhalinus* under the same environmental conditions to answer the question. Because there are some doubts in relation to the taxonomic problem whether the fewer vertebrae of *H. sakhalinus* may be a effect of some environmental conditions especially of low temperature. The material used for the observation was the eggs of 22 individuals of *H. olidus* and 12 individuals of *H. sakhalinus*. The eggs were attached to Petri dishes after fertilization, and then bathed in a glass tank. This tank was keeped cool with city water. The minimum temperature of the water of the tank was 5.5°C and the maximum 11°C, usually 7° to 9°C. Plate VII, Fig. A shows the embryo of *H. olidus* and Fig. B shows that of *H. sakhalinus*. Although morphological difference was not found in the two species, the embryo of *H. olidus* hatched in 22 to 35 days, mostly in 30 days and over (Pl. VIII). On the other hand the embryo of *H. sakhalinus* hatched in 19 to 34 days and the most part in 20 to 25 days (Pl. IX).

An increase in the number of segments is also associated with protracted development having a basis in a great increase in the amount of yolk in the egg (Hubbs 1926). In this connection, the eggs of *H. olidus* and *H. sakhalinus* were fixed in Bouin's solution, dehydrated with alcohol and dried in the air in order to compare the weight of egg of the two species. The eggs were weighed by means of torsion balance per every 200 in number and their average weight was calculated. As the result of the examination, the egg of *H. sakhalinus* is found to be significantly more weighty than *H. olidus*. Namely, the egg of the former is 0.035 mg in weight and that of the latter is 0.030 mg. It was ascertained that the embryo of *H. sakhalinus* hatched in shorter time than that of *H. olidus* under the same condition in spite of the more weighty egg. The difference of the weight between eggs of *H. olidus* and *H. sakhalinus* is almost certainly due to the difference of the amount of yolk in the egg.

Under the natural conditions (in Lake Ishikari-Furukawa), the embryo of *H. sakhalinus* hatches in about 18 days. The water temperature of the spawning bed of the lake is 9.7°C to 11.3°C and the chlorinity is 35.5 mg/L during the period of the development of the embryo (April 19 to May 7). *H. olidus* hatched in about 25 days in Lake Onuma. The water temperature there shows 0.5° to 18°C and the chlorinity is 2.9 mg/L during the development of the embryo (April 8 to May 2).

The taxonomic problems between *H. olidus* and *H. sakhalinus* might be solved by the fact stated above. The writer will quote here the opinion of Th. Dobzhansky (1955) : "If the populations live in the same country, sympatrically, and yet intermediates between them do not occur, they are almost certainly isolated reproductively."

3) *Hypomesus japonicus*

The ecology of *H. japonicus* has been almost unknown until now. Taranetz, A. (1936) described the spawning habit of *H. pretiosus* (=*H. japonicus*) in his taxanomic paper. According to his description, *H. pretiosus* spawns near the shore of the sea where water is somewhat low in salinity. They do not ascend to spawn in the river, and live along the coast with *H. olidus* in the summer and autumn.

Text-figure 5 (samples 30 to 41) shows the body length frequencies of *H. japonicus*. Sample 30 obtained from the coast of Mori on Sept. 14, 1954 may represent a school composed of individuals of the same age. No scale reading was carried out, for the reason of the desqumation of the scales in the greater part of the specimens of this sample. Similarity was observed in samples 31, 32, 33 and 34. The frequency of the body length of sample 35 obtained from the coast of Mori on April 20, 1955, leads to the presumption that the sample is composed of various age groups. One annulus was read on the scales of 21 specimens and two annuli were observed on those of 2 specimens (143 mm, 156.8 mm in body length).

According to Hamada, K. (1956), 1 age fish obtained from the coast of Kiritappu were separated from 0 age fish by the difference of the body length, and the scale clearly showed the age. However, sample 35 was not divided into two groups by difference of the body length; moreover, there was no annulus on the scales of the four specimens which were 120 to 124 mm in body length. Therefore, there is a doubt about the age determination by the scale reading. The scale reading was not carried out in sample 36 for the reason of the desquamation of the scales. In sample 37, one annulus was observed on the scales of three specimens (Plate IV, fig. b). There are two specimens which show one annulus on their scales in sample 38 obtained from the coast of Hakodate on April 4, 1954. It has been shown by the narrow variation of the body length that the specimens in this sample are the same age. This indicates that the annulus observed on the scale of two specimens may be a pseudoannulus. Sample 39 is composed of 0 age fish, for the frequency distribution of the body length shows one mode and that range is narrow, additionally no annulus is found on their scales. In the specimens of sample 40 obtained from Lake On-neto (salt water) in December 1953, there were two specimens which were found to possess one annulus on a scale (125.3 mm, 147.1 mm in body length) and two specimens (163.8 mm, 175.6 mm in body length) with two annuli. The specimens of sample 41 obtained from Lake On-neto taken on February 10, 1956 are too small to be *H. japonicus* which is found in February. This school may be a different race, for such small size fishes were never found before in Lake On-neto. There was no annulus on their scales.

The writer wishes to review samples 30 to 37, obtained from the coast of Mori. These samples were arranged in sequence of date of collection in Text-fig. 5. It is noticeable in the figure that the body length increases, as days go by, from sample 30 to 36, though the increase is no clear between samples 33 and 34. On the specimens of sample 30, it is evident that the school is composed of 0 age fish which hatched in the spring of 1954. The rightness of this con-

clusion was previously proved by K. Hamada (1956). Sample 35 shows the growth of *H. japonicus* for the preceding twelve months. Therefore, *H. japonicus* matures in a year, and dies after spawning with some exceptions.

On the spawning habit of *H. japonicus,* Taranetz, A. (1936) reported that they spawn in the water of low salinity near the seashore. His report is the only one on the spawning habit of *H. japonicus.* In Japan, there is no direct observation on that activity, though it has been presumed that they spawn maybe near the shore of the sea where some freshwaters pour in.

The writer examined carefully the surf of Fujimi-cho, Mori, Hokkaido, and on May 3, 1957 succeeded in discovering a spawning ground of *H. japonicus.* In that spot the writer discovered fortunately many eggs attached to grains of sand (Plate VI, Figs. a and b). Plate V (a, b, c and d) shows the coast where the writer found the spawning ground. The salinity of the water in the spawning ground was 25.083‰ and the water temperature 11°C. The low salinity is due to the fresh water of about twenty streams which pour into beach. When the eggs were found, the waves were beating upon the surf. If the sea is calm, the salinity of the sea water of the spawning ground may be more affected by the fresh water. Forty-eight eggs were found at the depth of about 0.5 m. Of them, 15 eggs were preserved in 70% alcohol after being fixed in Bouin's solution. Thirty-three eggs were reared in the water which was carried by train from the spawning ground to the laboratory. All eggs hatched at 10° to 17°C in 15 to 21 days (Plate VI, Figs. a, b, c and d). The egg is very similar to that of *H. olidus* in the characters as Okada, S. (1957) stated. But the egg-membrane is somewhat opaque, and the myotomes of the embryo were not observed through the membrane. According to the fishermen's observation, the spawn begins at about three hours after the ebb-tide. This habit may be related to their habit of spawning the eggs on the sand in the surf.

According to Okada, S. (1957), the eggs of *H. japonicus* are fertilized in the water of salinity 26‰ and less, and those of *H. olidus* are fertilized in the water of salinity 10‰ and less. Namely, the water of the spawning ground in the coast of Mori is fit for the fertilization and hatching of *H. japonicus.*

In spite of the writer's supreme effort, he failed to observe *H. japonicus* which is spawning. But according to fisherman's observation, a male and a female situate side by side, and poke their heads into sands in a tremor.

VI. Comparative Review of the Taxonomic Problems and the Ecology

There are, in some respects, different opinions on the classification in the species which are near relative from the view point of the systematic biology. The differences of opinion may be due to insufficiency of ecological observation and consiedration. If a species is discussed only from the view point of morphology, and if there is no remarkable difference of characters between that species and another, the question whether the species must be distinguished from each other or not, may be determined by subjective jadgment, to some extent.

a) *Hypomesus olidus* and *H. japonicus*

The number of the vertebrae and the lateral scales has been mentioned as a sharp difference of the characters between *H. olidus* and *H. japonicus*. It has been admitted generally however that the number of vertebrae and other segmentally arranged structures in fishes are influenced by the environmental conditions, particularly by temperature (Hubbs 1922). This possibility of the variation in vertebral number gave some authors doubt that *H. japonicus* may be a synonym of *H. olidus*. Hamada, K. (1954) reported difference of the number of vertebrae and the lateral scales as a remarkable difference of the characters between *H. olidus* and *H. japonicus*, and further pointed out the differences in the form of the stomach, the number of pyloric caeca and the length of maxillary. Of these characters, the number of vertebrae is most influenced by the environmental conditions as described above, and according to Kafuku, T. (1952) the digestive tract of the wild goldfish is not always invariable.

As stated before, one of the doubts on the classification of the two species is due to the lack of detailed observation on the spawning of *H. japonicus* until now. However, *H. japonicus* spawns undoubtedly in the surf, as was observed by the writer, and does not ascend into the fresh waters. And contrariwise, *H. olidus* spawns in the fresh waters and never spawns in the sea. Some individuals of *H. japonicus* ascend in the river Abashiri in the autumn, but this is a reasonable case because the sea water flows backward into the river. The spawning ground of the two species differs; *H. japonicus* never mingles with *H. olidus* for the purpose of the reproduction. The writer separates *H. japonicus* from *H. olidus* as different species for the reasons as stated above and in consideration of Dobzhansky's opinion that species may be kept apart by various reproductive isolating mechanisms, not only by hybrid inviability and sterility.

b) *Hypomesus sakhalinus, H. olidus, H. olidus bergi* and *H. olidus drjagini*

As stated before, *H. sakhalinus* is distinguished sharply from *H. olidus* by various differences such as the form of the air-bladder, the length of the interorbital width relative to the length of the snout, the length of the maxillary relative to the length of the head and the number of pyloric caeca. Moreover, *H. sakhalinus* does not descend into the sea even if there is a passage thereto, while *H. olidus* does descend, on the contrary, into the sea. It is remarkable that in *H. sakhalinus* the position of the connection from the pneumatic duct to the air-bladder differs from that of *H. olidus*. In this case, there is the question whether *H. sakhalinus* is isolated from *H. olidus* for reproduction or not. In Lake Ishikari-Furukawa *H. sakhalinus* spawns about four weeks before the spawning of *H. olidus* which ascends from Ishikari Bay. The ascending fishes spawn in the waters about 28 km up the river. That is to say, the two clearly do not mingle for the purpose of reproduction. *H. olidus* is also found in Lake Ishikari-Furukawa all the year round; the ecological difference is not distinct between the two species at present, excepting that *H. sakhalinus* does not descend into the sea. Then, it is considered that *H. sakhalinus* may be a resident as is observed in *Oncorhynchus masou*. However, *H. sakhalinus* does not shown the character of young as the parr mark which is observed in the

resident (land-locked form) of *O. masou*; the air-bladder of *H. sakhalinus* shows more developed form in comparison with that of *H. olidus* and other fishes belonging to Gen. *Hypomesus*. For the reasons stated above, the writer separated *H. sakhalinus* from *H. olidus**.

The writer has had no chance of examining *H. olidus bergi* and *H. olidus drjagini*. According to Taranetz, A. (1937), *H. olidus bergi* is differentiated from *H. olidus* by the larger eye, the existence of two pyloric caeca and the smaller size of body. This subspecies is found only in the small lake situated by the river Tuim, northern Sakhalin. That lake lacks any river emptying into the sea. Consequently, *H. olidus bergi* is not able to descend to the sea, that is, it is land-locked. This lake is poor in food for fish, and *H. olidus bergi* feeds on *Diptera*. Excepting *H. olidus bergi*, *Pungitius* sp. is the only one fish inhabiting the lake. If *H. olidus bergi* which inhabits such specific water, is given the normal environmental conditions, it is reasonable to expect it to show similar characters with *H. olidus*, though the writer has no good datum for a discussion of the point. There is only one fact which attracts the writer's attention. It is that *H. olidus* land-locked in Lake Ikeda is similar to *H. olidus* obtained from the other lakes in characteristics in spite of the cruelly lean body and the small size of the body. *H. olidus drjagini*, which was found in the basin of the Koluma River, was separated by Taranetz, A. (1937) from *H. olidus* by the difference of the number of pyloric caeca. According to his description, this subspecies has always two finger-like pyloric caeca, while typical *H. olidus* has always two finger-like pyloric caeca on the right side of the stomach and one on the left side. However, according to the writer's investigation, the pyloric caeca of *H. olidus* are four to seven in number as stated before; Andriyashev, A. P. (1954) stated that *H. olidus* has two to four pyloric caeca. The difference between the writer's observation and Andriyashev's in number means that the number of pyloric caeca varies with the localities. Consequently, the writer can not but reach the definite conclusion that the separation of *H. olidus drjagini* from *H. olidus* by only the difference in number of pyloric caeca is erroneous.

c) *Hypomesus japonicus* and *H. pretiosus*

The difference of the taxonomic characteristics between these two species is recognized in the number of vertebrae and lateral scales. Without this difference, there is no morphological characteristic to distinguish the species from each other. In ecology, there may also be no difference. Both species spawn in the surf. Their distribution however is segregated. Namely, *H. japonicus* is distributed in the region from Japan to the Arctic shores of North America. On the contrary, *H. pretiosus* occurs along ocean beaches from southern Alaska

* Lake Ishikari-Furukawa is the semicircular lake which was formed by the artificial change of the water way of the river Ishikari in 1931. *H. olidus* was transplanted into this lake from Lake Abashiri. *H. olidus* inhabiting the lake at present may be the offspring of the transplanted specimens. Formerly, before the transplantation of fish, the fishing of pond smelt was carried on in only the period of ascending through the passage from the sea.

to central California. The writer has not observed *H. pretiosus* in detail, though he seen the specimen so named by American ichthyologists. Taranetz, A. (1936) regarded *H. japonicus* as the synonym of *H. pretiosus*. Between *H. japonicus* and *H. pretiosus*, there may be no evident difference in the taxonomic characteristics nor in ecology. The writer follows Hubbs's opinion, however, at present and classifies *H. japonicus* as different from *H. pretiosus*, for he has no the good data to refute Hubbs's opinion.

VII. Problems of Speciation

The creation of a new species or new genus of sexually reproducing animals, through the branching of an evolutionary line, requires that a portion of the original common stock become isolated, so that its interbreeding with the rest of the stock is prevented or greatly restricted. The kinds of barriers which seem to prevent interbreeding between existing species can (for purposes of the present account) be classified as: geographical, that is, separation by physical features; ecological, that is, separation by differences in habitat; temporal, that is, separation by time of breeding; and generative, that is, separation by differences in the behaviour associated with reproduction, by nonconformity of generative organs, by incompatibility of gametes, or by differences in chromosomal mechanism (F. Neave, F. R. S. C. 1958).

The ecological divergence found in *Oncorhynchus nerka* and *Salmo gairdneri* attracts the attention of the ecologist, in relation to the problems of speciation or the evolution (W. E. Ricker 1938, 1940, F. Neave 1944). Through his studies of Gen. *Hypomesus*, the writer came to the conclusion, as the cause of speciation, that the ecological divergence which arises in relation to the environment is one of the causes of speciation, though F. Neave (1958) holds the converse opinion that ecological divergence is often the result rather than the cause of speciation.

As stated already, *H. olidus* may be divided into the a-type of anadromous form, the b-type of anadromous form and the land-locked form. Some of *H. olidus* belonging to the b-type of anadromous form remain in the fresh water through their life, even when they have the way to descend into the sea. The writer named them "residual-type of anadromous form", and distinguishes them from the land-locked form which is prevented from the descending into the sea by physical factors. Accordingly, *H. olidus* is divided into two forms and three types.

1) *a-type of anadromous form*
2) *b-type of anadromous form*
3) *residual type of anadromous form*
4) *land-locked form*

There is no difference in the morphological characters among the b-type of anadromous form, the residual type of anadromous form and the land-locked form, excepting a difference in size of the body. Namely, morphological difference is not found among these forms, though there are ecological differences among them. On the other hand, the a-type of anadromous form differs from

the other forms in the larger numbers of vertebrae and lateral scales, and in that the spawning period is later than the other forms, as stated before. There is no mingling of individuals for the purpose of reproduction betwen the a-type of anadromous form and the others. That is, the a-type of anadromous form is isolated from the others for reproduction, while, the isolation is very imperfect between the b-type of anadromous form and the residual type of anadromous form. Differences of spawning period and spawning ground are not found between the latter two, so far as the present investigation is concerned. As stated above, differences of some characteristics are found between the a-type and the b-type of anadromous form, but such are not found between the b-type of anadromous form and the residual type. Nevertheless the land-locked form inhabits limited waters as a microcosm; it is not distinguished from the b-type of anadromous form nor from the residual type in morphological characters. The ecological difference between the a-type of anadromous form and the b-type of anadromous form or the residual type of anadromous form is not a difference which occurs under physical compulsion. As stated above, there are several races in *H. olidus*. How or when these races were formed is an important and interesting problem in relation to the evolution of animals.

According to Oshima, M. (1957), *Oncorhynchus rhodurus* inhabits the fresh waters during their life, but the descending *O. rhodurus* is found merely in the adjacent waters near the mouth of the river Kiso. Oshima described how this species requires abundant animal food and the low water temperature as the inhabitable conditions, while the salinity of the habitat may have no importance among the conditions for inhabiting.

The writer considers that the race of *H. olidus* results from ecological divergence which originates in the relation to environment. The important factors of the environmental conditions may be the water temperature and the feed. The density of fish population, ~~may be related to~~ the feed and the water temperature may be the reason that part of *H. olidus* migrate to sea and the other part remain in the lake as is observed in *H. olidus* inhabiting Lake Abashiri. That the progenies of *H. olidus* which were retarded in growth and the development are prevented from descending into the sea with the rapid rise of the water temperature of the epilimnion is possible. The physiological change of the fish is undoubtedly important, but it is not so important that the need of salt water controls the death-and-life of *H. olidus*, for it becomes land-locked easily. It may be arosed by the want of feed, or by the high temperature in the summer that the anadromous *H. olidus* does not remain in the river Ishikari. The environment might have worked continually upon *H. olidus* during geological time. The natural selection which works upon them, certainly increased their ecological and morphological divergence. This divergence may have caused differentiation of the species. Four species belonging to *Hypomesus* must have been originated through the process as stated above.

VIII. Problems of Fishery

At present, *H. olidus* is transplanted artificially into many lakes and is

stocked in the sea for the purpose of propagation. It is a great success that *H. olidus* has been transplanted to unexploited lakes and bred artificially or naturally in the waters which it was compelled to inhabit. For example, *H. olidus* of Lake Onuma transplanted from Lake Abashiri has rated commercially an important place. It may be however more effective, if *H. sakhalinus* is transplanted instead of *H. olidus*, for the former adapts successfully to the fresh waters in comparison with *H. olidus*. The a-type of anadromous form is fit to stock in the river, for it descends into the sea and ascends in the river after attaining growth. The b-type of anadromous form is fit as stocking *H. olidus* in the lake which has the way to the sea, for this form descends to the sea and lives there about six months, then comes back to the lake.

The writer has some doubts on the effect of the stocking of *H. olidus* in the sea. *H. japonicus* were obtained from the coast of Mori in large number by the writer, but *H. olidus* was not found. Many fishermen think that the increase of the population of *H. japonicus* along the coast of Mori is the result of the stocking of *H. olidus*. That however is erroneous; *H. japonicus* is a different species from *H. olidus* as stated before. Nevertheless it is not evident why the population increased recently; it is considered to be one of the reasons that fresh water empties increasingly along that seashore as the result of the cutting down of the forest trees and for others reasons. In general, a flowing of fresh water in seashore maybe exert bad influence upon spawning grounds of fishes, while *H. japonicus* requires, however, the flowing of the fresh water for their spawning as stated before. This special habit of the spawning of *H. japonicus* is thought to be one of the reasons of the increase of the population.

IX. Summary

From 1947 until 1960 the writer carried out taxonomic and ecological studies of Gen. *Hypomesus* which is distributed in the lakes and the coast of Japan. In this paper, he proposes solutions to some problems in the taxonomy and the ecology, and moreover he referred to the problems of speciation and the fishery.

The materials used in the present studies are listed in tables 1 to 5.

ON THE TAXONOMY *H. olidus* is sharply separated from *H. japonicus* in smaller number of the vertebrae, there being 52 to 59, less than 60 to 67; large maxillary, 36 to 50% of head length, longer than 30 to 39%; usually in the more anterior position of the ventral fins in reference to the dorsal, the ventral insertion being in most cases in advance of, instead of a little behind, the vertical from the origin of dorsal fin.

H. sakhalinus is distinguished from *H. olidus* in small length of snout, the snout being shorter, instead of longer, than interorbital width; in small length of maxillary, 29 to 40% of head length, less than 36 to 50%; smaller number of pyloric caeca, there being 1 to 3, sometimes lacking, less than 3 to 7; pneumatic duct inserted about 1/5 distance from tip of air-bladder, instead of tip of air-bladder.

H. japonicus differs from *H. sakhalinus* in large number of vertebrae and

pyloric caeca, and in position of connection of pneumatic duct to air-bladder as stated above.

H. pretiosus is distinguished from *H. japonicus* in small size of scales, there being 66 to 76 (C. Hubbs, 1925).

It is somewhat doubtful to separate *H. olidus bergi* and *H. olidus drjagini* from *H. olidus,* for the large eye and small size of the body are perhaps resulted from special environment, and small number of pyloric caeca of *H. olidus bergi* may be a local variation.

ON THE ECOLOGY *H. olidus* is separated into two forms and three types by their ecologies as follows:

A) *anadromous form*
 1) *a-type of anadromous form*
 2) *b-type of anadromous form*
 3) *residual type of anadromous form*
B) 4) *land-locked form*

The a-type of anadromous form inhabits the sea, and ascends into a river to spawn in the spring. It matures, spawns and dies in a year. The b-type of anadromous form ascends into a lake in the autumn after having grown in the sea from spring to autumn, and spends the winter. It matures in a year, and spawns on the shore of a lake or flowing river. Some of this form escape death and spawn again in the next year. A few of them are alive for four years. The residual type of anadromous form does not descend into the sea, but remains in the fresh waters throughout their life. However, it is not different essentially from the b-type of anadromous form from the ecological point of view, for individuals of the residual type and the b-type mingle for the purposes of reproduction. The land-locked form is the form which is not able to descend to the sea by reason of some physical obstacle as the case of *Oncorhynchus nerka* found in Lake Akan, Hokkaido. This form does not differ essentially from b-type of anadromous form from the ecological point of view.

H. sakhalinus has similar life-history to the residual type of anadromous form of *H. olidus.* It does not descend into the sea; it spawns near the margin of a lake.

H. japonicus matures in a year, and spawns along the seashore in the spring. The waters of the spawning ground are somewhat low in salinity, for the fresh water streams find outlet there. The water temperature is about 11°C. The eggs attach to grains of sand. The characters of their eggs are similar to those of *H. olidus,* but the egg membrane is somewhat opaque.

Some authors question whether *H. japonicus* may be a synonym of *H. olidus.* However, *H. japonicus* spawns in the surf and does not ascend into fresh waters. *H. olidus* and *H. japonicus* do not mingle for the purpose of reproduction. From the facts as stated above, it is evident that *H. olidus* and *H. japonicus* are species which differ from each other.

PROBLEMS OF SPECIATION The writer considers that the race of *H. olidus* results from ecological divergence which originates in the relation to the environment. The important factors of the environmental conditions may

be the water temperature and the feed. In the past, the environment exerted continual influences upon *Hypomesus* during and since geological times; natural selection occurred. Temporal isolation for reproduction arose surely between the races. The natural selection which exerted influences upon them, certainly increased their ecological and morphological differences. The different species must have been originated through such process.

The a-type of anadromous form of *H. olidus* is isolated from other forms by its different ecology; it has different characteristics. On the other hand, there is no difference in morphological characters among the other three, the b-type of anadromous form, the residual type of anadromous form and the land-locked form. This shows the completion of ecological isolation from other forms and the beginning of differentiation of form that the a-type of anadromous from has both different ecology and different characteristics of the body. There is no difference of the characters of the body among the b-type, the residual type and the land-locked form, for they are not completely isolated.

PROBLEMS OF FISHERY *H. olidus* is widely transplanted into the lakes and the ponds. However, *H. sakhalinus* may be most fit for transplanting into the lakes, for it adapts itself thoroughly to the fresh waters in comparison with *H. olidus.* The a-type of anadromous form is fit as the form of *H. olidus* to be transplanted to a river for it ascends in the river to spawn after having grown. The b-type of anadromous form ascends to a lake from the sea in the autumn, while some of them remain in the lake through their life. Therefore this form of *H. olidus* is fit for transplanting into the lakes which have a way to the sea.

Recently, *H. olidus* is stocked in the coast of Uchi-ura Bay as one method of propagtions. There are however some doubts in the effect, for the greater part of the smelt caught in the bay is *H. japonicus,* not *H. olidus.* The reason of the increase of the smelt, *H. japonicus,* is not evident, but it is considered as one of the reasons that fresh water flows increasingly along the seashore as the result of the cutting down of the forest trees and the like. *H. japonicus* requires the inflow of the fresh water for its spawning. This special habit of *H. japonicus* is thought as one of the reasons of the increase of the population.

Bibliography

Amemiya, I. & Y. Hiyama (1940). Wakasagi no sanran oyobi nenrei ni tsuite. *Suisan-gaku-kaiho,* 8 (1), 45–62 (in Japanese).

Andriyashev, A. P. (1954). Fishes of Northern Seas of USSR. *Acad. Sci. USSR,* 113–115 (In Russian).

Berg, L. S. (1932). Les poisons des eaus douces de l'U. R. S. S. et des limitrophes. *3rd Ed., Pt.* 1, 284–285 (In Russian).

Brevoort, J. C. (1856). Notes on some figures of Japanese fish. *Narr. Comm. M. C. Perry's Exp. to Japan,* 2, 278.

Clark, F. N. (1929). The life history of the California jack smelt, *Atherinopsis californiensis. Divis. Fish and Game Calif., Fish Bull.* 16, 1–23.

Dobzhansky, Th. (1951). Genetics and the Origin of Species. *3rd Edition. Columbia University Press, New York.* 1-348.

——— (1955). Evolution, Genetics, and Man. *John Wiley & Sons, INC. New York.* 1-398.

Evermann, B. W. & E. L. Goldsborough (1906). The fishes of Alaska. *Bull. Bur. Fish.*, 24, 269-270.

Guenther, A. (1866). Catalogue of the fishes in the British Museum. 6, 169-170.

Hamada, K. (1953a). Ecological studies of the pond smelt, *Hypomesus olidus* (Pallas). Rep. 1. On the local difference of the scale structure. *Bull. Japan. Soc. Scien. Fish.*, **19** (2), 75-78 (in Japanese).

——— (1953b). Ecological studies of the pond smelt, *Hypomesus olidus* (Pallas). Rep. 2. On the pond smelt of the old and the pre|sent water ways of the river Ishikari. *Bull. Japan. Soc. Sci. Fish.*, **19** (2), 79-82 (in Japaneses).

——— (1953c). Ecological study of the pond smelt, *Hypomesus olidus* (Pallas). Rep. 3. Uncertainty of age determination by the scale structure on the pond smelt obtained from Lake Abashiri, and the reason therefore. *Bull. Fac. Fish. Hokkaido University*, **4**, (1), 46-53 (in Japanese).

——— (1956a). Ecological study of the pond smelt, *Hypomesus olidus* (Pallas). Rep. 4. On the age of the pond smelt ascending in the Ishikari River. *Bull. Fac. Fish. Hokkaido University*, 7 (3), 213-218 (in Japanese).

——— (1956b). Ecological study of *Hypomesus japonicus*. Rep. 1. Conclusion as to age. *Bull. Fac. Fish. Hokkaido University*, 7 (3), 219-224 (in Japanese).

——— (1954). A revision of *Hypomesus olidus* (Pallas) and *Hypomesus japonicus* (Brevoort) of Hokkaido, Japan. *Bull. Fac. Fish. Hokkaido University*, **4** (4), 256-267.

——— (1957). A New Osmerid Fish, *Hypomesus sakhalinus* new species, Obtained From Lake Taraika, Sakhalin. *Japan. Jour. Ichthy.* 5 (3/4/5/6), 136-142.

Hubbs, C. L. (1922). Variations in the number of vertebrae and other meristic characters of fishes correlated with the temperature of water during development. *The American Naturalist*, **56**, 360-372.

——— (1925). A revision of Osmerid fishes of the North Pacific. *Proc. Biol. Soc.* Washington, 38, 49-56.

——— (1926). The structural Consequences of Modifications of the Developmental Rate in Fishes, Considered in Reference to Certain Problems of Evolution. *Amer. Nat.* **60**, 58-81.

——— (1934). Racial and individual variation in animals, especially fishes. *Amer. Nat.* 57, 115-128.

——— (1940). Speciation of fishes. *Amer. Nat.* 124, 198-211.

Jordan, D. S. & B. W. Everman (1896). The fishes of North and Middle America. *Bull. U. S. Nat. Mus.*, 47 (1), 519-527.

——— & C. H. Gilbert (1883). Synopsis of the fish of North America. 294-295. Washington.

——— & ——— (1899). The fishes of Bering Sea. *Fur-Seal and Ful-Seal Islands of the North Pacific Ocean*, 3, 439-440.

——— & C. L. Hubbs (1925). Record of fishes obtained by David Starr Jordan in Japan, 1922. *Mem. Carneg. Mus.*, 10 (2), 151-152.

——— & C. W. Metz (1913). A catalogue of fishes known from the waters of Korea. *Mem. Carneg. Mus.*, 6 (2), 11.

——— & J. O. Snyder, (1902). A review of the Salmonoid fishes of Japan. *Proc. U. S. Nat. Mus.*, 24, 567-593.

Kobayashi, H. (1936). Rinso ni yotte hanmei suru wakasagi no seitai. *Kagaku*, 6 (3), 100-102. (in Japanese).

Kafuku, T. (1952). Races of wild goldfish with special reference to the digestive tract. *Bull. Freshwater Fish. Res. Lab.*, 1 (1), 41–56. (in Japanese).

Nojima, S. (1938). Wakasagi to chika no sai ni tsuite. *Sake-Masu Iho, Sake-Masu Hogo Kyokai*, 36, 1–4. (in Japanese).

Neave, F. (1944). Racial characteristics and migratory habits in *Salmo gairdneri*. *J. Fish. Res. Bd. Canada*, 6, 245–251.

——— (1958). The Origin and Speciation of *Oncorhynchus*. *Trans. Roy. Soc. Canada*. Ser. III, 52, Sec. 5, 25–39.

Oshima, M. (1957). Studies on the dimorphic Salmons, *Oncorhynchus masou* (Brevoort) and *Oncorhynchus rhodurus* Jordan & McGregor, found in Japan and adjacent Territories. *Japan. Jour. Ichthy.*, 5 (3/4/5/6), Supplement, 1–79 (in Japanese).

Okada, S. (1957). Wakasagi to chika no seitaiteki-sai ni-san. *Hokkaido Sake-Masu Fukajo. Uwo to Tamago*, 8 (6), 34–44 (in Japanese).

Pallas, P. S. (1811). Zoographia Rosso-Asiatica. 3, 391.

Ricker, W. E. (1938). "Residual" and kokanee salmon in Caltus lake. *J. Fish. Res. Bd. Canada*, 4 (3), 192–218.

——— (1945). On the Origin of Kokanee, A Fresh-water type of Sockeye Salmon. *Trans. Roy. Soc. Canada, Ser.* III, 34, Sec. 5, 121–135.

Schmidt, P. U. (1904). Pisces marium orientalium imperii rossici, 281–282 (in Russian).

——— (1950). Fishes of Okhotsk Sea. *Bulletin of Pacific Commission*, 6, 47-49 (in Russian).

Soldatov, V. K. & G. L. Lindberg, (1930). A review of the fishes of the seas of the Far East. *Bull. Pac. Sci. Fish. Inst.*, 5, 59–60.

Sano, S. (1937). Abashiri-Ko no omonaru seisoku-gyo ni tsuite. *Sake-Masu Iho*, 4 (34), 7–16. (in Japanese).

Sato, R. (1950). Biological observation on the pond smelt, *Hypomesus olidus* (Pallas), in Lake Kogawara, Aomori Prefecture, Japan. 1. Habits and age composition of the spawning fishes. *Tohoku Jour. Agr. Res.*, 1 (1), 87–95.

——— (1951). Influence of natural environmental conditions of the vertebral number of the pond smelt, *Hypomesus olidus* (Pallas). *Tohoku Jour. Agr. Res.* 2 (1), 127–133.

Shiraishi, Y. (1952). Study on spawning migration of pond smelt, *Hypomesus olidus* by marking in Lake Suwa. *Bull. Freshwater Fish. Res. Lab.* 1 (1), 26–40 (in Japanese).

———, Kitamori, R. & Kitamori, M. (1955). The growth of pond smelt, *Hypomesus olidus* in Lake Suwa and its lepidological study. *Bull. Freshwater Fish. Res. Lab.* 4 (1), 17–32 (in Japanese).

Taranetz, A. (1936). Freshwater fishes of the basin of the north-western part of the Japan Sea. *Trav. l'Inst. Zool. l'Acad. Sci. l'USSR.*, 4, 497–499 (in Russian).

Taranetz, A. (1937). Materials on the study of ichthyofauna of Soviet Sakhalin. *Bull. Pac. Sci. Inst. Fish. Ocean.*, 15–17 (in Russian).

Explanation of Plates

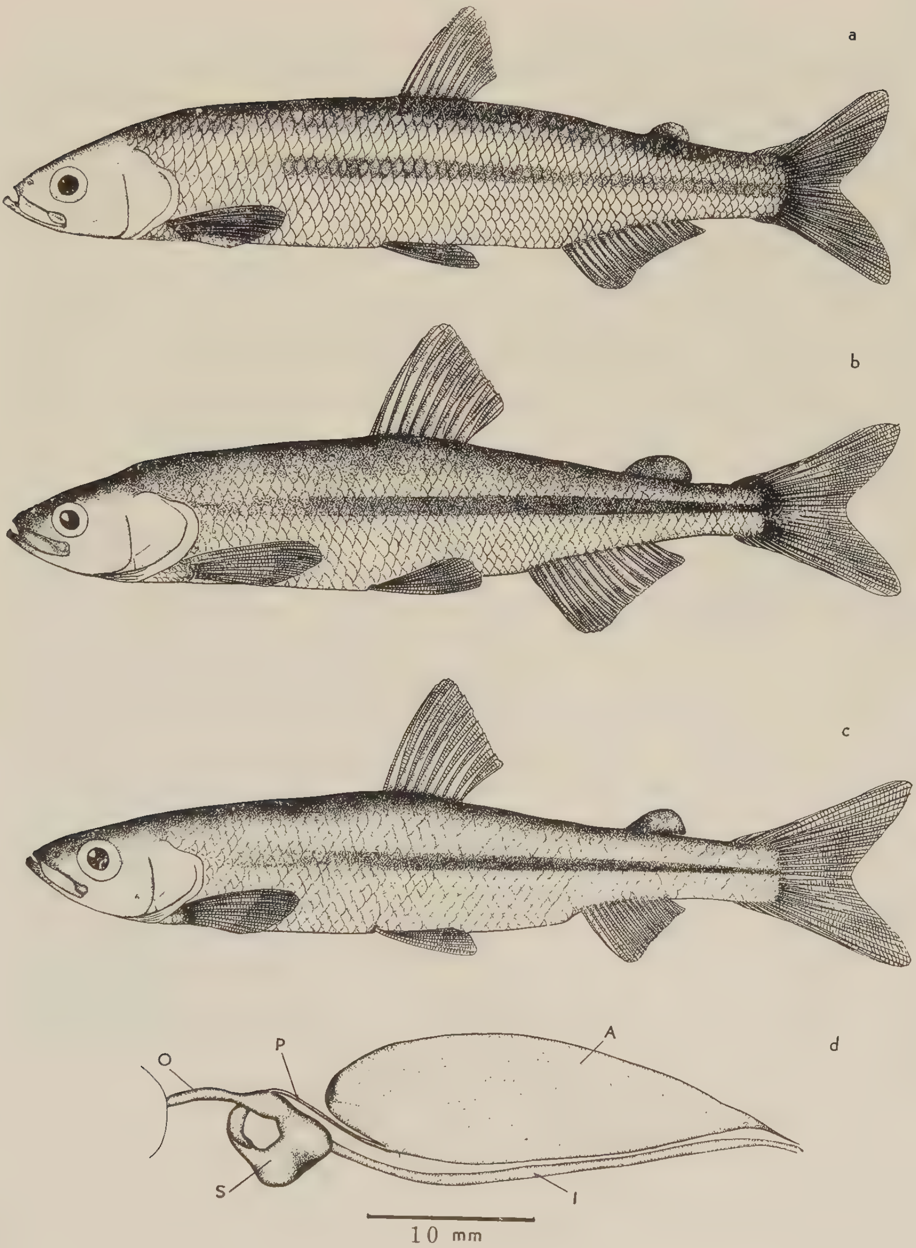
PLATE I

a: *Hypomesus olidus* obtained from the river Ishikari on May 27, 1949. Body length 102.4 mm, male fish.

b: *Hypomesus sakhalinus* obtained from Lake Ishikari-Furukawa on April 24, 1950. Body length 94 mm, male fish.

c: *Hypomesus sakhalinus* obtained from Lake Ishikari-Furukawa on April 24, 1950. Body length 78 mm, female fish.

d: Air-bladder of *H. sakhalinus* obtained from Lake Ishikari-Furukawa on April 24, 1950. Body length 79 mm, male fish.



K. Hamada: Taxonomic and Ecological studids of *Hypomesus*

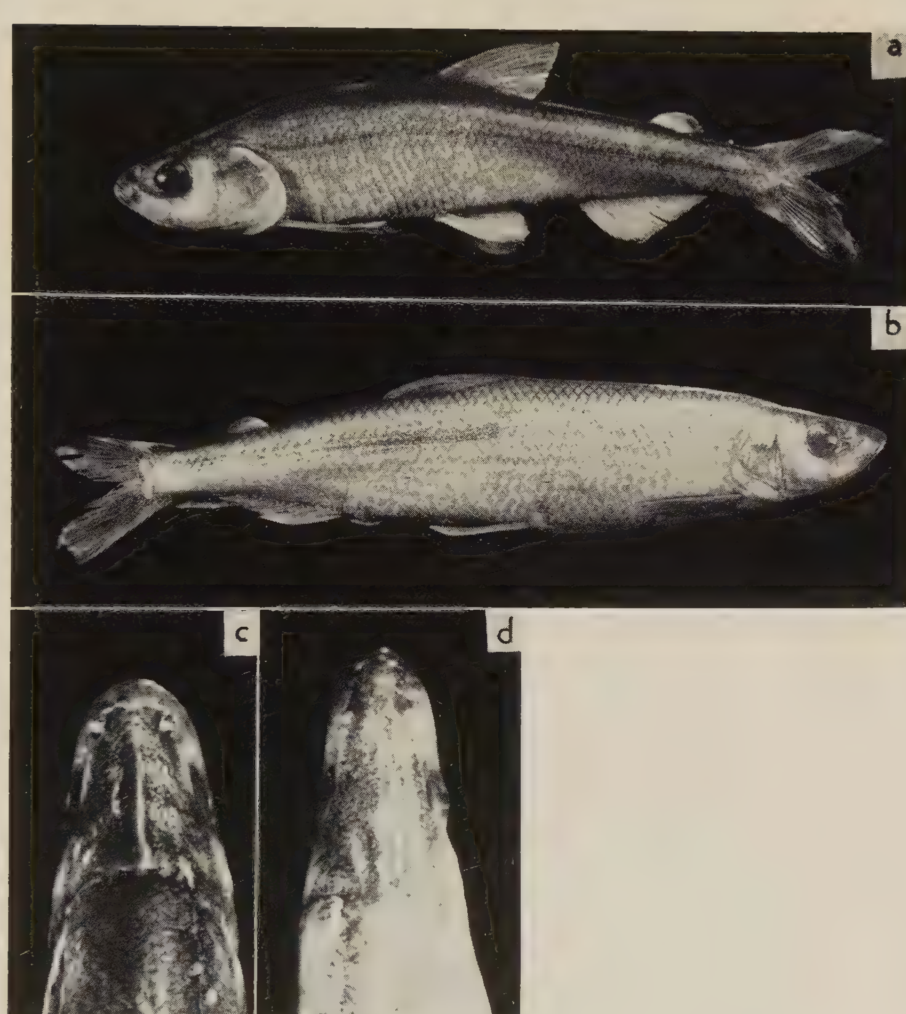
PLATE II

a: *Hypomesus sakhalinus* obtained from Lake Ishikari-Furukawa on April 24, 1950, Body length 111 mm, female fish.

b: *Hypomesus olidus* obtained from Lake Ishikari-Furukawa on April 24, 1950. Body length 125 mm, female fish.

c: Dorsal view of the head of the specimen in Fig. a.

d: Dorsal view of the head of the specimen in Fig. b.



K. Hamada: Taxonomic and Ecological studies of *Hypomesus*

PLATE III

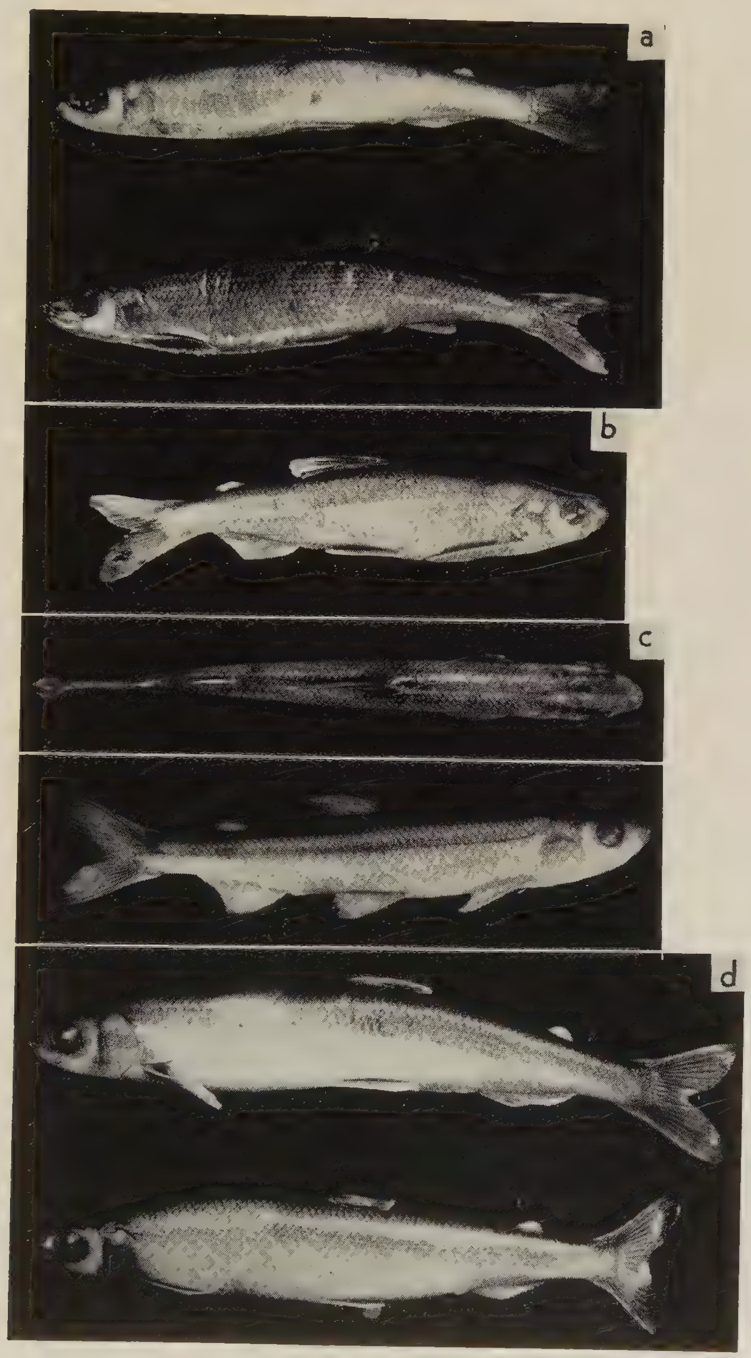
a: Above; *H. olidus* obtained from Lake Ishikari-Furukawa on April 18, 1950, Body length 90 mm, male fish.
Below; *H. olidus* obtained from Lake Ishikari-Furukawa on April 18, 1950. Body length 95 mm. female fish.

b: *H. sakhalinus* obtained from Lake Ishikari-Furukawa on April 18, 1950. Body length 82 mm. male fish.

c: *H. sakhalinus* obtained from Lake Ishikari-Furukawa on April 20, 1953. Body length 84.7 mm, female fish. This fish was reared in an artificial pond for 25 weeks and 3 days. Above, dorsal view; below, side view.

d: *H. japonicus* obtained from the coast of Hakodate on April 10, 1954. Above; male fish, body length 126.7 mm. Below; female fish, body length 116.2 mm.

PLATE III

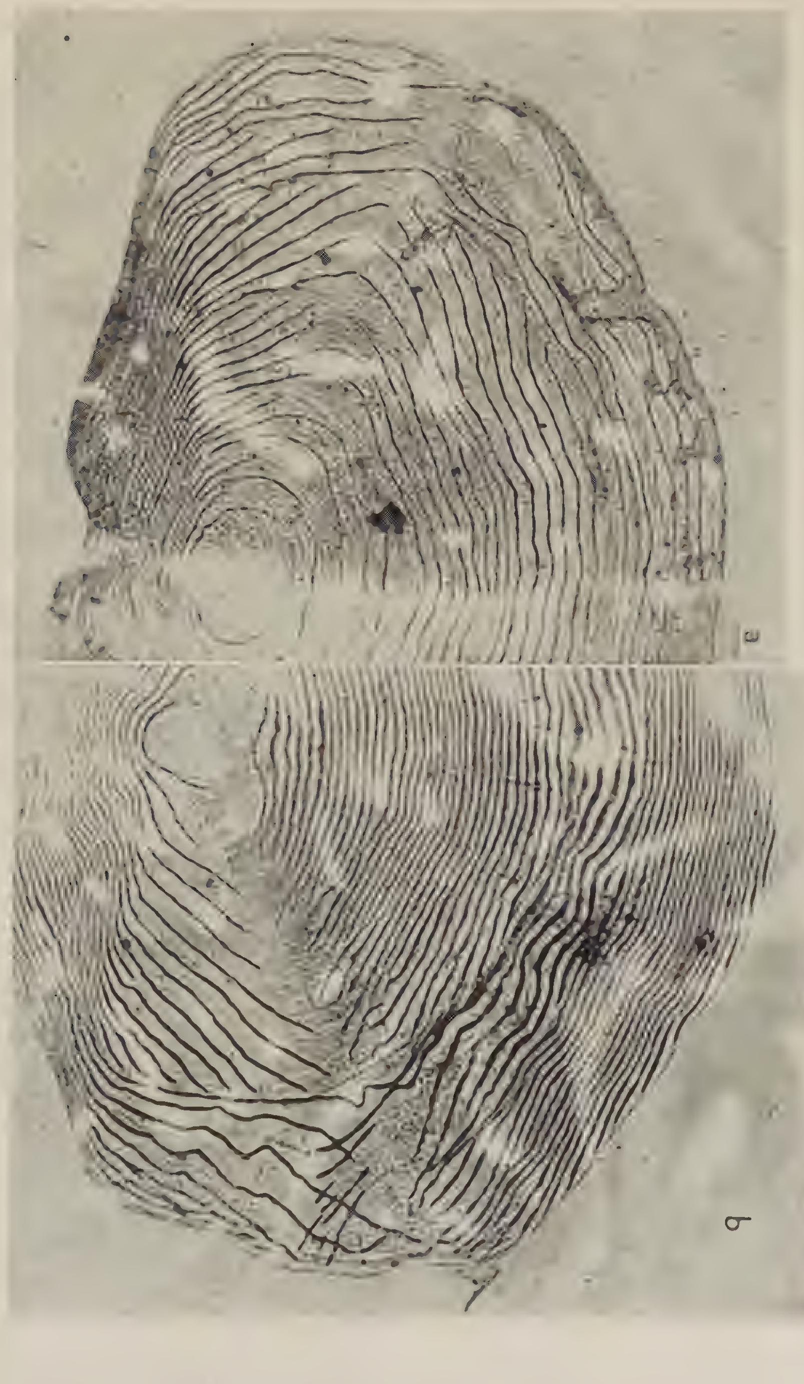


K. Hamada: Taxonomic and Ecological studies of *Hypomesus*

PLATE IV

a: Scale of *H. sakhalinus*, showing three annuli, obtained from Lake Ishikari-Furukawa on April 24. 1950. Body length 111 mm, female.

b: Scale of *H. japonicus*, showing one annulus, obtained from the coast of Mori on May 6, 1957. Body length 155.8 mm, female.



K. Hamada: Taxonomic and Ecological studies of *Hypomesus*

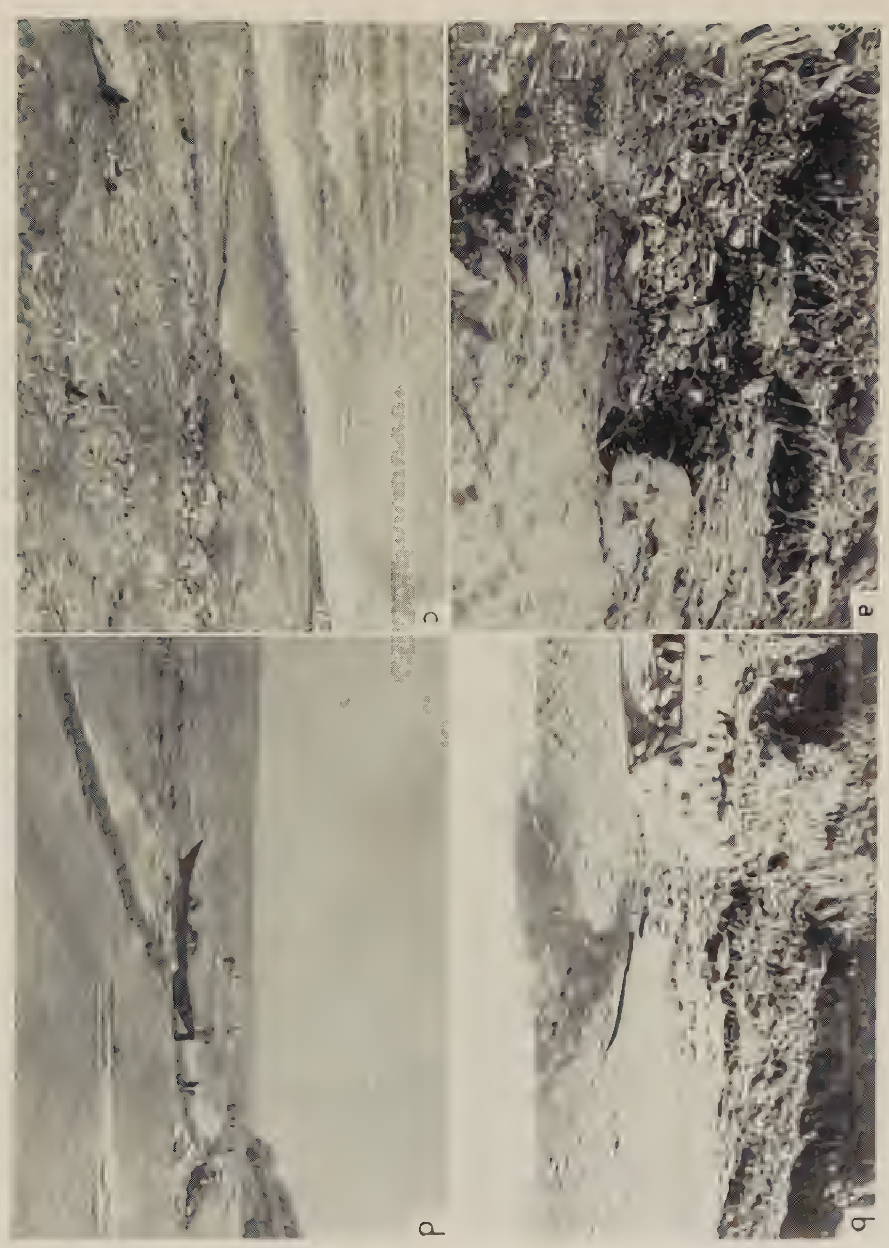
PLATE V

a: Ground of the well of the fresh water emptying on to the seashore.

b: Stream of fresh water emptying on to the seashore. The stream has a width of about 1 m.

c: Beach at the spawning ground of *H. japonicus*.

d: Catching of the smelt, *H. japonicus*.

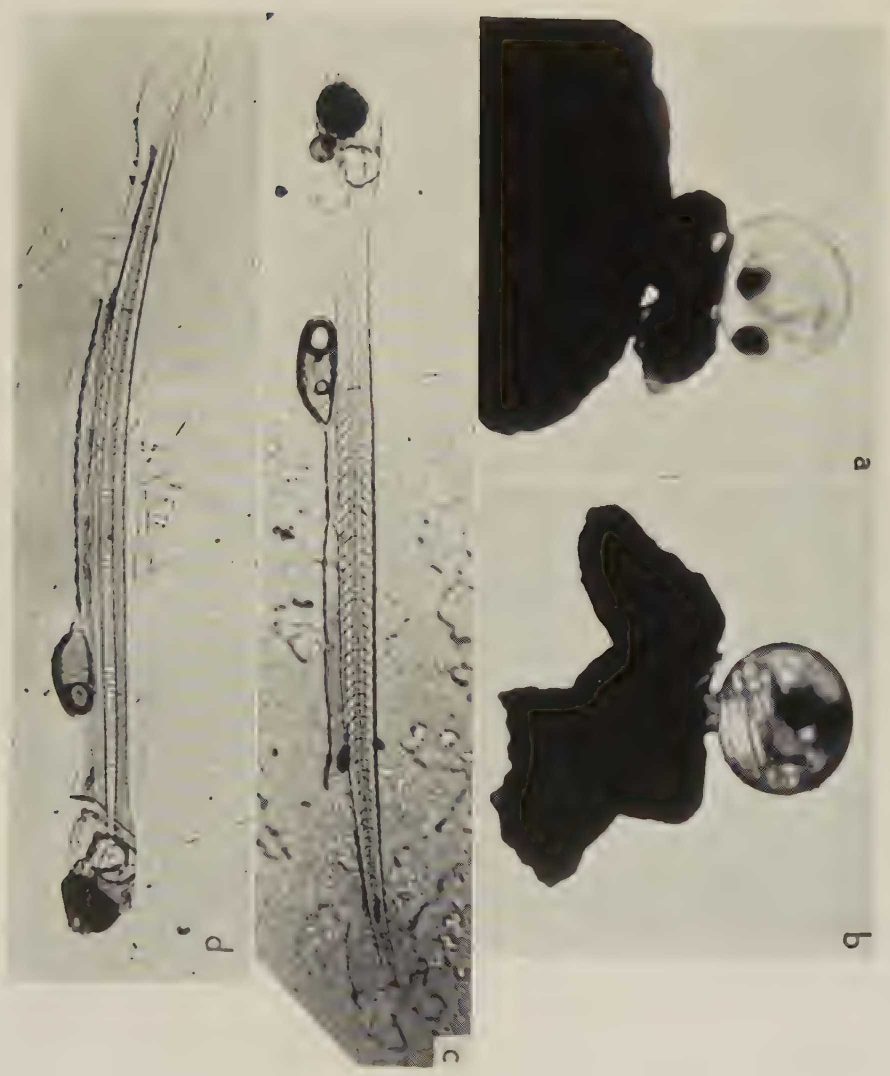


K. Hamada Taxonomic and Ecological studies of *Hypomesus*

PLATE VI

a and b: Eggs of *Hypomesus japonicus*, found at the beach of Mori on May 3, 1957. Water temperature 11°C. Photograph on May 13, 1957. Twenty × size of the original.

c and d: Embryo of *Hypomesus japonicus* at about 3 hours after hatching. Twenty × size of the original.



K. Hamada: Taxonomic and Ecological studies of *Hypomesus*

PLATE VII

Photomicrographs of the embryos of *H. olidus* (A) and *H. sakhalinus* (B).

Fig. A.

1. one-cell ovum, 8 hours.
2. two-cell ovum, 8 hours.
3. four-cell ovum, 8 hours.
4. early gastrula, 24 hours.
5. gastrula, 72 hours.
6. expansion of the forebrain, 6 days.
7. heart beating, 15 days.
8. formatoin of otolith, 17 days.
9. pigmentation of ventral margin, 22 days.

Fig. B.

1. two-cell ovum, 5 hours.
2. four-cell ovum, 8 hours.
3. early gastrula, 16 hours.
4. middle gastrula, 24 hours.
5. closure of blastopore, 5 days.
6. expansion of brain, 7 days.
7. formation of cavity in optic vesicle, 8 days.
8. pigmentation in optic vesicle, 11 days.
9. heart beating, 12 days.
10. pigmentation of ventral margin, 15 days.

PLATE VII

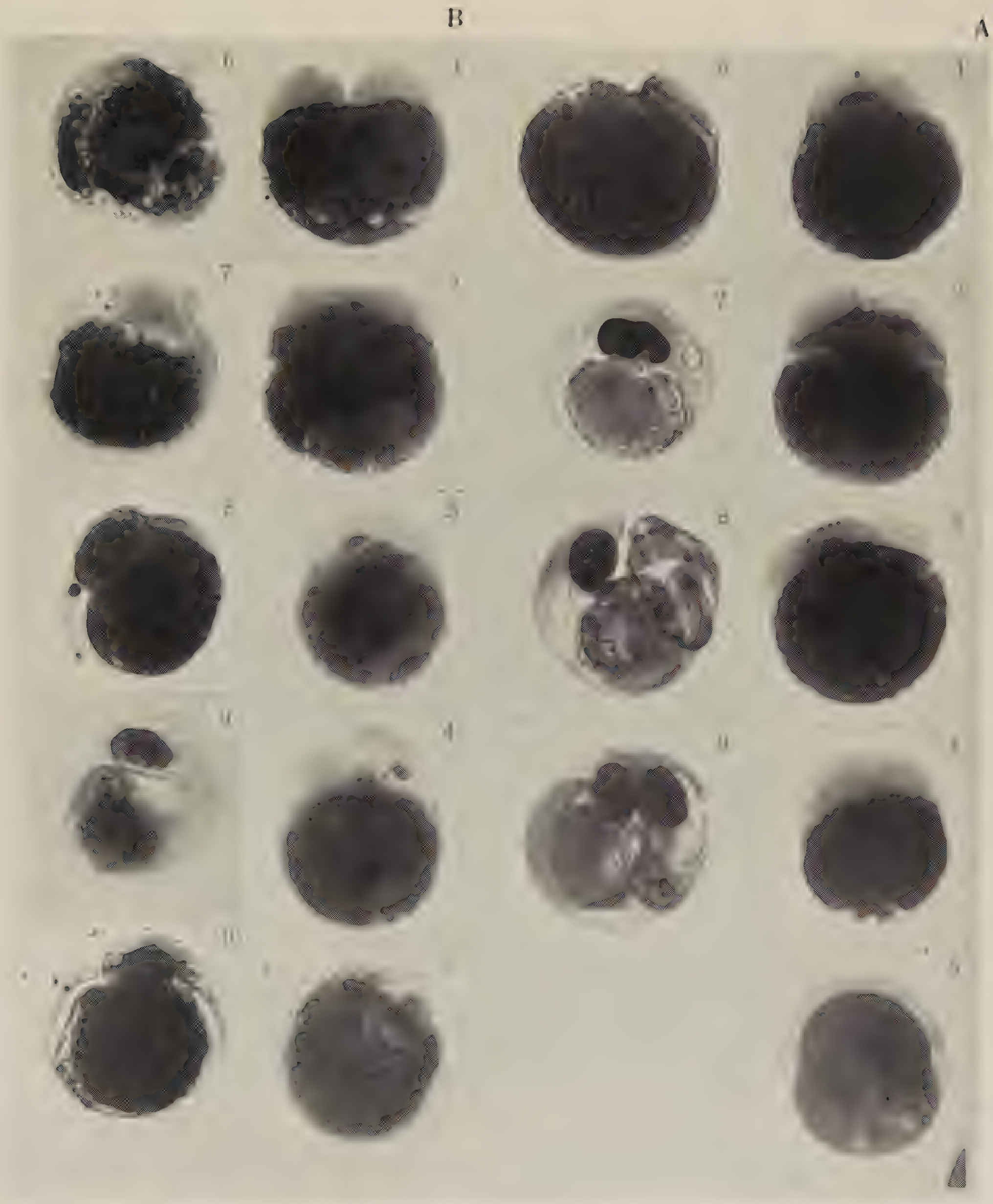


PLATE VIII

Photomicrographs of the embryos of *H. olidus* just after hatching.

a: embryo hatched on the 35th day, water temperature 9°C, maximum 11.3°C, minimum 5.5°C.

b: embryo hatched on the 35th day, water temperature 9°C, max. 11.3°C, min. 5.5°C.

c: embryo hatched on the 15th day. These were hatched in course of carriage from Lake Onuma to the laboratory by the train. The pectoral fin not formed.



K. Hamada: Taxonomic and Ecological studies of *Hypomesus*

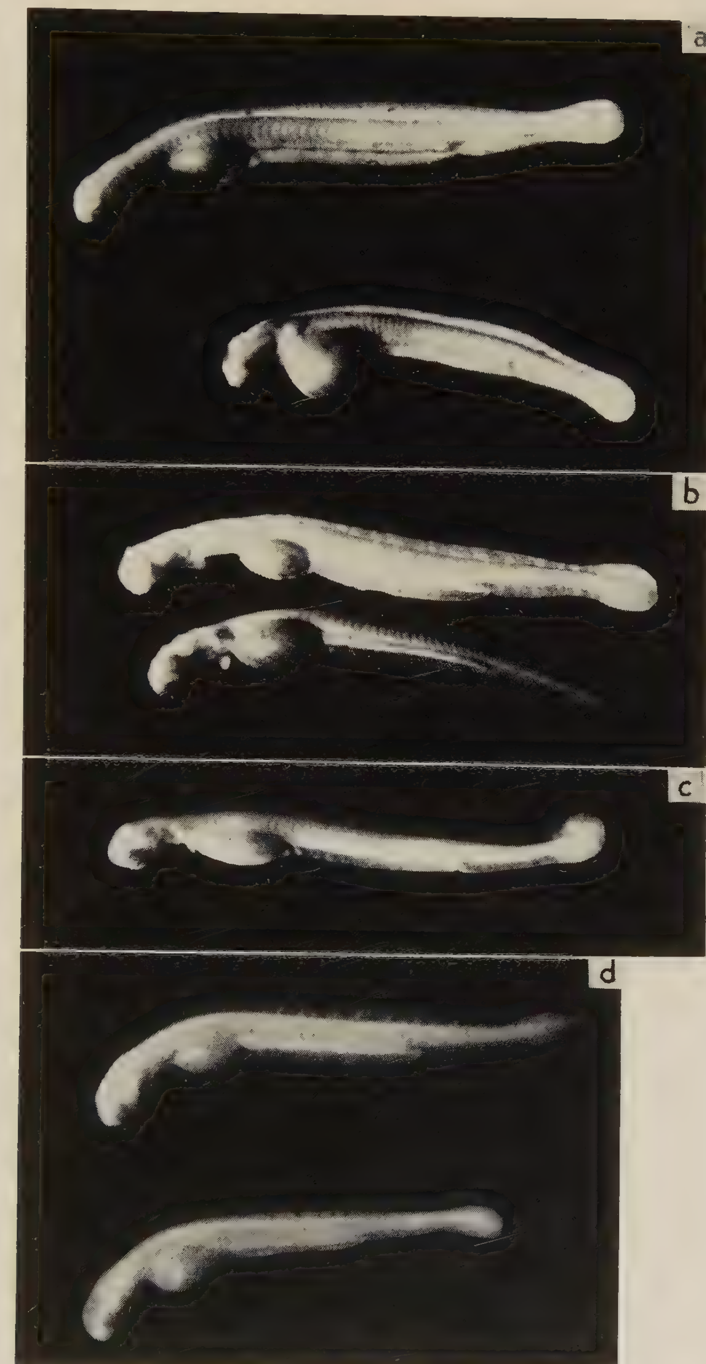
PLATE IX

Photomicrographs of the embryos ~~of the embryos~~ of *H. sakhalinus* just after the hatching.

a: embryo hatched on the 22~~th~~nd day, water temperature 8°C. max. 11.3°C, min. 5.5°C.

b: embryo hatched on the 28th day, water temperature 9.5°C. max. 11,3°C, min. 5.5°C.

c and d: embryo hatched on the 29 th day, water temperature 9.5°C, max. 11.3°C. min. 5.5°C.



K. Hamada: Taxonomic and Ecological studies of *Hypomesus*

SQUID TROPOMYOSINS

SHUICHIRO KUBO

SQUID TROPOMYOSINS

SHUICHIRO KUBO

Faculty of Fisheries, Hokkaido University, Hakodate, Japan

Contents

Introduction

Tropomyosin, which is one of the structure proteins of muscle fibril, was first discovered by Bailey[1] in 1946, and crystallized by him[2] from rabbit skeletal muscle dehydrated with organic solvents. The physico-chemical and chemical characteristics have been observed in detail on rabbit muscle tropomyosin[2-9]: it is an asymmetric protein and belongs to the α-keratin-epidermin-myosin-fibrinogen group according to the x-ray diffraction pattern. Its amino acid composition also resembles that of myosin. On the other hand, tropomyosin differs from actin not only in its behavior towards salts, but also in its amino acid composition.

Tropomyosins can be prepared in high purity more easily than other structural proteins in muscle. They have been prepared from different types of mammalian muscle[10-14], from lower classes of vertebrata[15-21] and from invertebrata[11-12,14,22-32]. They can be distinguished from other proteins by their high contents of acidic and basic amino acids. The ratio of lysine and arginine contents was found to change regularly with the phylogenetic level of the animal origin. They have been classified on the basis of their solubility and the ratio of lysine and arginine contents as water-insoluble and -soluble tropomyosins[27] or tropomyosin A and B[25,29].

Squid tropomyosin has already been isolated by Yoshimura from the mantle of *Ommastrephes sloani pacificus* STEENSTRUP[22], by Tsao et al. from *Sepia esculenta*[12,14] and by Kominz et al. from *Loligo pealeii*[29]. According to the amino acid composition and the diagram of salting-out with ammonium sulphate, all these tropomyosins belong to the water soluble tropomyosin or tropomyosin B.

In the present study, two kinds of crystalline proteins have been isolated from the tropomyosin fraction in the mantle of *Ommastrephes sloani pacificus* STEENSTRUP. Their solubility, amino acid composition, terminal residue and molecular size and shape have been elucidated. From these results, it is concluded that the one is identical to Yoshimura's tropomyosin or tropomyosin B whilst the other belongs to the water-insoluble or tropomyosin A group.

Acknowledgments

The author wishes to express his heartiest thanks to Professor Y. Ogura, Professor T. Ando and Professor H. Noda, Faculty of Science, Tokyo University, Tokyo, for their valuable criticisms of this study. He is greatly indebted to Professor K. Yoshimura, Faculty of Fisheries, Hokkaido University, Hakodate, and to Professor Y. Tonomura, Research Institute for Catalysis, Hokkaido University, Sapporo, for their invaluable advice in improving the manuscript and their constant encouragement during the investigation. The author's cordial thanks are also due to Professor S. Akabori and his collaborators, Faculty of Science, Osaka University, Osaka, for their instruction in the technique of amino acid analysis and terminal assay, and to Dr. T. Nihei, Department of Biochemistry, Dartmouth Medical School, Hanover, U.S.A., for his help in carrying out

the light-scattering measurements.

Experimental Results

I. Preparation of Protein

Bailey[24,27] developed the following two kinds of methods to extract and isolate tropomyosin from invertebrate muscle. The one is the extraction of tropomyosin from minced muscle, which was dehydrated beforehand with organic solvents, and the other the direct extraction from fresh minced muscle without dehydration, both being followed by the crystallization of tropomyosin by dialysis against KCl solution of low ionic strength. The former method was more convenient than the latter on squid muscle for the sake of the highly viscous actomyosin. In order to separate the two crystalline tropomyosins obtained in this study, it was, however, necessary to combine the dialysis method with the salting-out technique, since the two tropomyosins showed similar solubility in KCl solution of low ionic strength.

1) Methods

Isolation: Step 1. The muscle of fresh squid mantle, of which the cuticle has been rejected, was cut down to 10~20 mm width and was washed with ice cold water. The frozen muscle pieces, kept overnight at about −20°C, were passed through a meat mincer. This procedure was convenient for homogenizing the muscle. Step 2. The minced muscle was put with occasional stirring in 2 volumes of cold 0.05 M KCl-0.005 M K_2HPO_4-KH_2PO_4 solution at pH 6.8 and 0°C for 30 min. to remove the soluble proteins, and was centrifuged at 1,000×g. Step 3. After addition dropwise of 3 volumes of ethanol, which was cooled at −10°C, the residue was suspended for 30 min. at 0°C. Ethanol washing two times was followed by two ether washings. The drained residue from ether was allowed to dry at room temperature for about 2 hours. Step 4. The dehydrated fiber was extracted with 10 volumes of cold M KCl-0.05 M K_2HPO_4-KH_2PO_4 solution at pH 7 and 0°C. After standing for 24 hours, the suspension was pressed through cheese-cloth. The residue was re-extracted with the M KCl buffer for 4 hours and the liquid was squeezed out. The combined solution was dialysed thoroughly against 0.1 M KCl-0.005 M K_2HPO_4-KH_2PO_4 solution at pH 6.5 and 0°C. Crystals of needle shape thus obtained are shown in Fig. 1. Step 5. After the addition of 1% celite, the dialysed solution was filtered through on a Büchner funnel. The filter cake was extracted with stirring in 10 volumes of M KCl-K_2HPO_4-KH_2PO_4 solution at pH 7.5 for 4 hours. The solution of extracted protein was clarified by filtration whereupon 0.25 volume of saturated ammonium sulphate solution, which was kept cold and adjusted to pH 7.0, was added gradually. The precipitate was filtered with celite on a Büchner funnel. Step 6 (a). The cold saturated ammonium sulphate solution was added slowly to the clear filtrate in portions up to 39% saturation. The precipitate was collected, dissolved in 0.5 M KCl-K_2HPO_4-KH_2PO_4 solution at pH 7 and dialysed against the same solution. The salting-out procedure was repeated with this dialysed solution, and the 20~37% salting-out fraction was



Fig. 1. Crystals of Step 4



Fig. 2. Silky sheen of protein B

collected (fraction A). (b). The filtrate, which was obtained from the 39% salting-out fraction in Step 6 (a), was brought to 54% saturation. The precipitate was suspended in water, dialysed against the buffer, and once more subjected to precipitation at 41~54% saturation (fraction B).

Crystallization: Crystalline protein A. The precipitate of fraction A was dissolved in a small amount of 0.5 M KCl-0.005 M K_2HPO_4-KH_2PO_4 solution at pH 7.5. The protein solution was dialysed against 0.25 M KCl-0.005 M K_2HPO_4-KH_2PO_4 solution at pH 6.5, and the concentration of KCl of the dialysing solution was gradually decreased to 0.20, 0.15 and finally to 0.10 M. The protein was obtained as crystalline precipitates at the bottom in the cellophan bag. Recrystallization was carried out by repetition of the above procedure.

Crystalline protein B. After the filter cake of fraction B had been dissolved in water, the protein solution was dialysed progressively against 0.40-0.15 M KCl-0.005 M K_2HPO_4-KH_2PO_4 solution at pH 6.5. The crystalline protein deposited finally in 0.15 M KCl buffer solution. The crystalline protein was also obtained by the following procedure: cold distilled water was slowly added with constant stirring to about 1% protein solution in M KCl buffer at pH 7 and 0°C, and the solution was diluted to a final concentration of 0.15 M KCl. A silky sheen appeared as illustrated in Fig. 2, and crystals of needle-like shape deposited on the bottom after 24 hours at 0°C.

Salting-out analysis: The salting-out curves were obtained according to the method of Snellman and Tenow[33-34]. The protein solution was thoroughly dialysed against a buffer solution (0.5 M KCl-0.1 M K_2HPO_4-KH_2PO_4). Each 1 ml of the protein solution was added to 4 ml of the mixture of saurated ammonium sulphate solution and the buffer containing different amounts of saturated ammonium sulphate in a series. The mixtures were allowed to stand overnight at 0°C, and were filtrated. After washing and filling up to a constant volume

with the buffer solution, the extinction of the filtrate was measured at 260 and 277 mμ.

Dialysing analysis: Each 2 ml of the protein solution in 0.5 M KCl-0.1 M K_2HPO_4-KH_2PO_4 solution at pH 7.14 was dialysed at 0°C for 3 days against several change of the buffer solution, which contained K_2HPO_4-KH_2PO_4 at ionic strength 0.005 and KCl from 0 to 0.50 M. After washing and filling up to a constant volume with the buffer solution, the mixture was centrifuged at 15,000×g for 30 min at 0°C. The extinction of the supernatant was determined at 260 and 277 mμ.

2) Results

Bailey obtained pure tropomyosins from dehydrated adductor muscle of pinna and foot muscle of octopus by a procedure up to Step 4. In the case of squid, the crystalline protein obtained in Step 4 illustrated in Fig. 1 was, however, found to be contaminated with some impurities, even after several times re-crystallization. The results of salting-out and dialysing analyses on this crystalline protein in Step 4 are shown in Figs. 3 and 4. It is evident from Fig. 3 that the protein is composed at least of six components, viz., I 9~18%, II 18~37%, III 37~54%, IV 54~60%, V 60~69% and VI 69~75% saturated fraction. Since the extinction ratio at 277/260 mμ of fractions IV, V and VI is 0.69, 0.78 and 0.80, respectively, these fractions seem to be contaminated with rather large amounts of nucleic acid.

Though the fractionation by the dialysing procedure is not so definite as that by the salting-out, in the dialysing curve three components were observed,

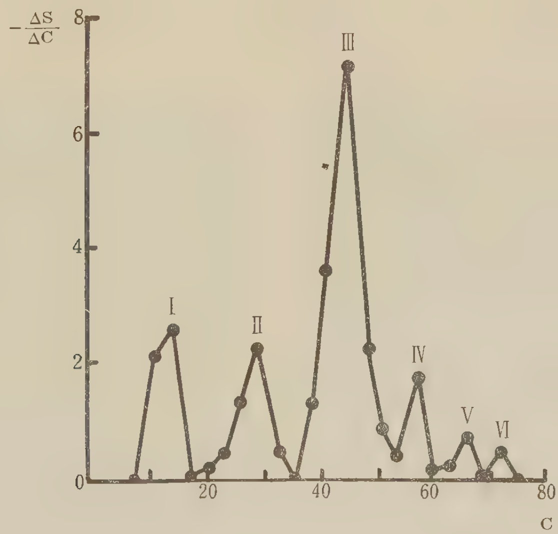


Fig. 3. Salting-out curve of the protein in Step 4; s, extinction at 277 mμ; c, per cent in volume of saturated $(NH_4)_2SO_4$

viz., 0.45~0.33 M KCl, 0.33~0.20 M KCl and 0.20~0.10 M KCl fraction*.

The chemical and physico-chemical properties of the proteins A and B will be described in the following sections.

II. General Properties of Crystalline Proteins A and B

In this section is described an examination of the purities of the crystalline proteins A and B by salting-out, electrophoretic and tryptophan analyses. The properties of the proteins will be described.

1) Methods

Salting-out and dialysing analyses: These methods have already been described above and applied to a protein solution of 0.5%.

Electrophoretic analysis: Electrophoresis was performed by a Hitachi Tiselius apparatus (HT-B type, cell size 2×15×50 mm) with a protein solution which was dialysed against a buffer (ionic strength 0.35 KCl and 0.1 K_2HPO_4-KH_2PO_4) at 0° for 48 hours.

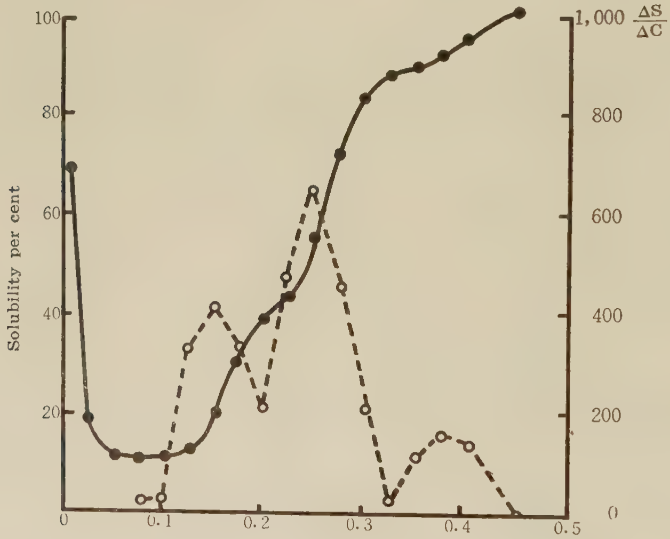


Fig. 4. Solubility curve of the protein in Step 4; s, soulubility per cent; c, concentration of KCl; ●, solubility; ○, $\Delta s/\Delta c$ Conditions: total protein concentration 1.02 %, pH 7.14, 0°C

* The protein solution in Step 4 was separated into two portions by the dialysis against 0.20 M KCl buffer, and two crystalline proteins were obtained from 0.20 M KCl and 0.20–0.1 M KCl fractions by the successive dialyses. The salting-out curves of the 0.20 M KCl and 0.20 –0.10 M KCl fractions revealed five and the three components, respectively.

The procedure described unnder "*Isolation*" gives the best yields of crystalline proteins A and B.

Total nitrogen content: The protein was suspended in 0.005 M acetate buffer at pH 5.0 and washed several times with the same buffer, then with water, dried in ethanol and ether, and finally dried at 110°C for 12 hours. After decomposition of the dried protein under the condition according to Miller[35], total nitrogen was determined by the micro-Kjeldahl method.

Tryptophan content: Tryptophan content was determined by the use of p-dimethylaminobenzaldehyde[36 37].

Ultraviolet analysis: The ultraviolet absorption spectrum of the protein solution (about 2 mg/ml in 0.35 M KCl and 0.1 M K_2HPO_4-KH_2PO_4) was measured by a Shimadzu spectrophotometer (QB type 50).

Total phosphorus content: Total phosphorus content was determined by the method of Allen[38].

2) Results

The crystalline proteins obtained from the fractions of 20~37% and 41~54% saturation are illustrated, respectively, in Figs. 5 and 6.

The salting-out curves of the crystalline proteins A and B are given in Figs. 7 and 8. The differential salting-out curves of the proteins A and B showed one sharp peak in the range of 21~39% and 40~54% saturation, respectively. These salting-out ranges agree, respectively, with those of tropomyosins A and B[25,29] or water-insoluble and water-soluble tropomyosin[27]. The precipitation ranges against KCl solution were below 0.25 M KCl and 0.35 M KCl, respectively, for the proteins A and B (Figs. 9 and 10). Below 0.025 M KCl both these proteins formed a swollen gel. The solubility of protein B in KCl solution was increased by decreasing pH to 4.0 and by increasing pH to 9.0, while the protein A denatured at pH below 5.4.

Each of the proteins was homogenous electrophoretically at pH 7.15 as illustrated in Fig. 11, showing a single peak with the mobility of descending 1.65×10^{-5} cm²/volt/sec and ascending 1.79×10^{-5} cm²/volt/sec for protein A and

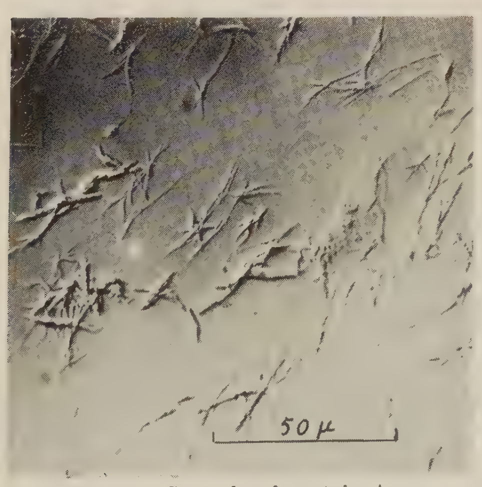


Fig. 5. Crystals of protein A (2 times of recrystallization)

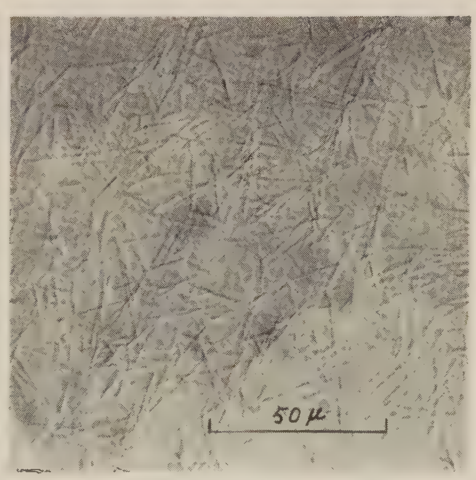


Fig. 6. Crystals protein B (2 times of recrystallization)

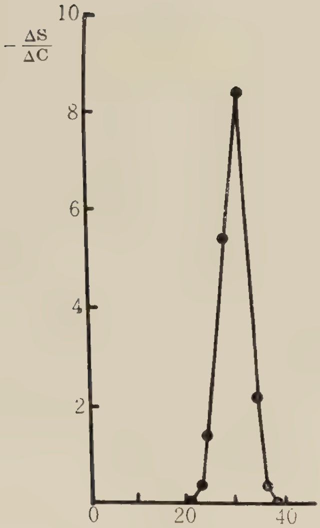


Fig. 7. Salting-out curve of protein A
s, extinction at 277 mμ; c, per cent in volume of saturated $(NH_4)_2SO_4$

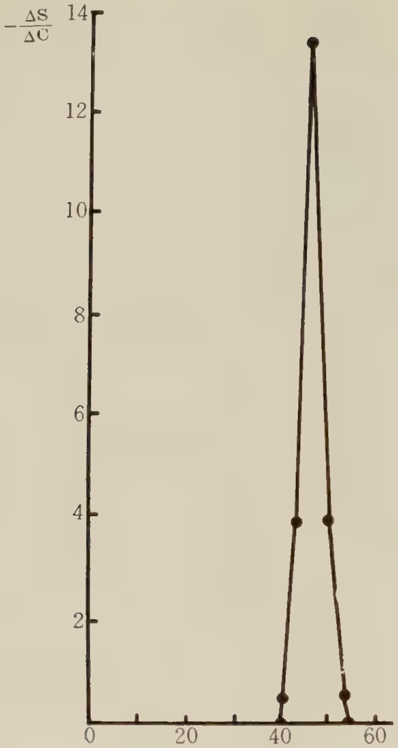


Fig. 8. Salting-out curve of protein B
s, extinction at 277 mμ; c, per cent in volume of saturated $(NH_4)_2SO_4$

of descending 2.10×10^{-5} cm^2/volt/sec and ascending 2.25×10^{-5} cm^2/volt/sec for protein B. The mobility of protein B was larger than that of A. This is consistent with the amounts of net negative charge estimated from their amino acid compositions.

The contents of total nitrogen, total phosphorus and tryptophan are recorded in Table 1. According to Bailey[2,27] tryptophan of the tropomyosin preparation is due to contaminations. The low contents of tryptophan (protein A 0.05, and protein B 0.02%) indicate the high purity of our preparations.

Both proteins showed the maximum absorption at 277 mμ and the minimum at 255 mμ, as given in Fig. 12. The extinction ratios of 277/255 mμ were 1.64 and 2.06, respectively for proteins A and B. The high values of extinction ratio of 277/255 mμ and their low phosphorus contents (protein A 0.06, and protein B 0.02%) indicate that these preparations are scarcely contaminated with nucleic acid.

III. Molecular Size and Shape of Proteins A and B

The size and shape of the proteins A and B were determined by the light-

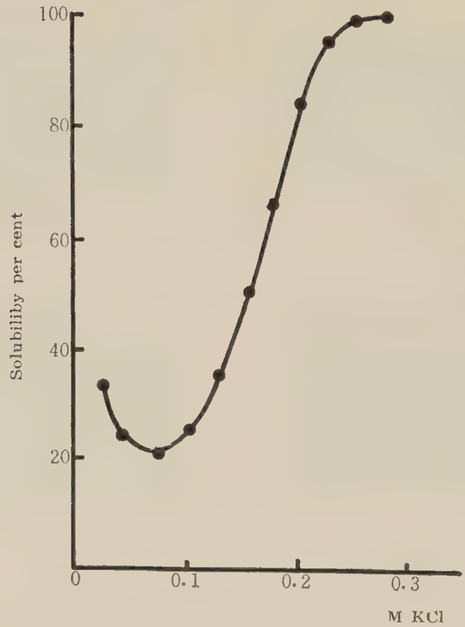


Fig. 9. Solubility curve of protein A; Conditions: total protein concentration 0.57 %, pH 7.14, 0°C

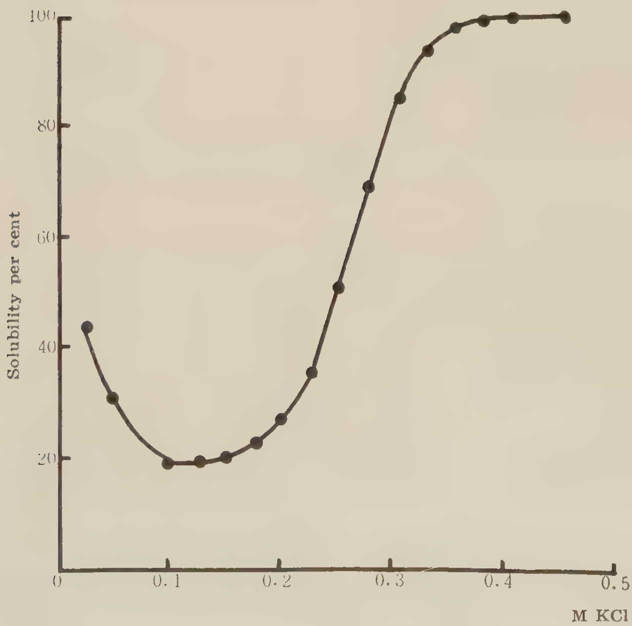


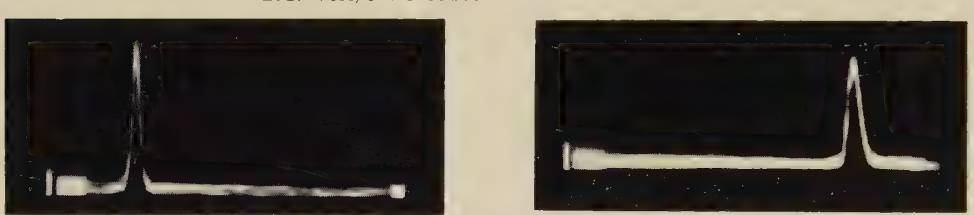
Fig. 10. Solubility curve of protein B; Conditions: total protein concentration 0.55 %, pH 7.11, 0°C

Protein A



Ascending Descending

I=0.45 (KCl 0.35 and K_2HPO_4-KH_2PO_4 0.1)
pH 7.15 protein concentration 0.57 %
2.47 volt/cm 8100 sec



Protein B

Ascending Descending

I=0.45 (KCl 0.35 and K_2HP_4-KH_2PO_4)
pH 7.15 protein concentration 0.70 %
2.47 volt/cm 8100 sec

Fig. 11. Electrophoretic diagrams of proteins A and B

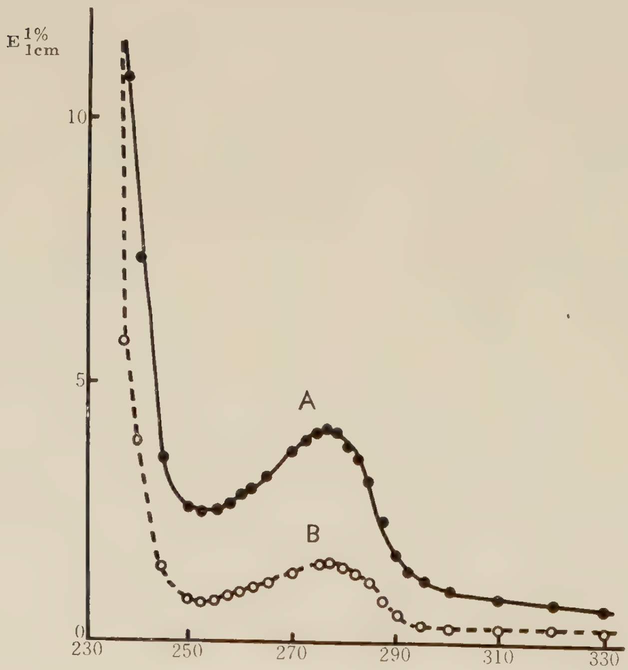


Fig. 12. Ultraviolet absorption curves of A and B; A, protein A; B, protein B; in ionic strength 0.45 KCl-K_2HPO_4-KH_2PO_4 solution

Table 1. Contents of total nitrogen, total phosphorus and tryptophan

Protein	Nitrogen (%)	Phosphorus (%)	Tryptophan (%)
Protein A	18.1	0.06	0.05
Protein B	18.1	0.02	0.02

scattering method. Tsao et al.[6] have found by observations of osmotic pressure and viscosity that the particle weight and viscosity increment of rabbit skeletal tropomyosin are remarkably affected by the ionic strength of solution and that the values of them at 0.6 M KCl reduce to about half by an increase of KCl concentration from 0.1 to 0.6 M.

Therefore, the intensities of light-scattering of the proteins A and B have been measured at high ionic strength of the solution. The size and shape of rabbit and fish (haddock) tropomyosins were also determined.

1) Methods

The protein solutions, dissolved in KCl-K_2HPO_4-KH_2PO_4 of ionic strength 1.25 (KCl 1.20 and buffer 0.05) at pH 7.05, were clarified two centrifugations at $10^5 \times g$, each for 1.5 hours, by means of a Hitachi preparative ultarcentrifuge. The light-scattering intensities of the protein solution were measured in a Brice-Phoenix apparatus at the wave length of 546 mμ and room temperature. The measurements of refractive index increment of the solutions, which were dialysed against the solvent, were carried out by means of a Brice-Phoenix differential refractometer under the same condition as the measurements of light-scattering. The values of the refractive index increment were 0.188, 0.187, 0.188 and 0.188 for squid A, squid B, rabbit and fish tropomyosins, respectively.

The molecular weight and the radius of gyration were derived from a graphical procedure of Zimm[39-40]: the molecular weight was given as the reciprocal of the intercept of the Zimm-plot with the $(Kc/R_\theta)c{=}o$, where R_θ is the intensity of light-scattering at the angle θ, and K is given by the following equation:

$$K=2\pi^2 n_0^2 (dn/dc)^2/N\lambda^4$$

The refractive index of the solvent is indicated by n_0, that of the solution by n, N is Avogadro's number and is the wave length of light in vacuum.

The square of the radius of gyration was obtained from the slope of the tangent which one was able to draw to the curve $(Kc/R_\theta)c{=}o$ as a function of $\sin^2(\theta/2)$ at the point where $\theta{=}o$.

Bcause of the presence of large aggregations, the intensities of light-scattering at low angles increased remarkably. Therefore, the intensities above 45° were adopted, to determine the molecular weight and the radius of gyration.

2) Results

In Figs. 13 and 14, the reduced intensities are presented as an angular envelope respectively for the proteins A and B. In Table 2, the molecular weight and radius of gyration are listed, together with those of rabbit and fish tropomyosins.

Both the molecular weight (125,000) and the length (rod 1,560 Å, coil

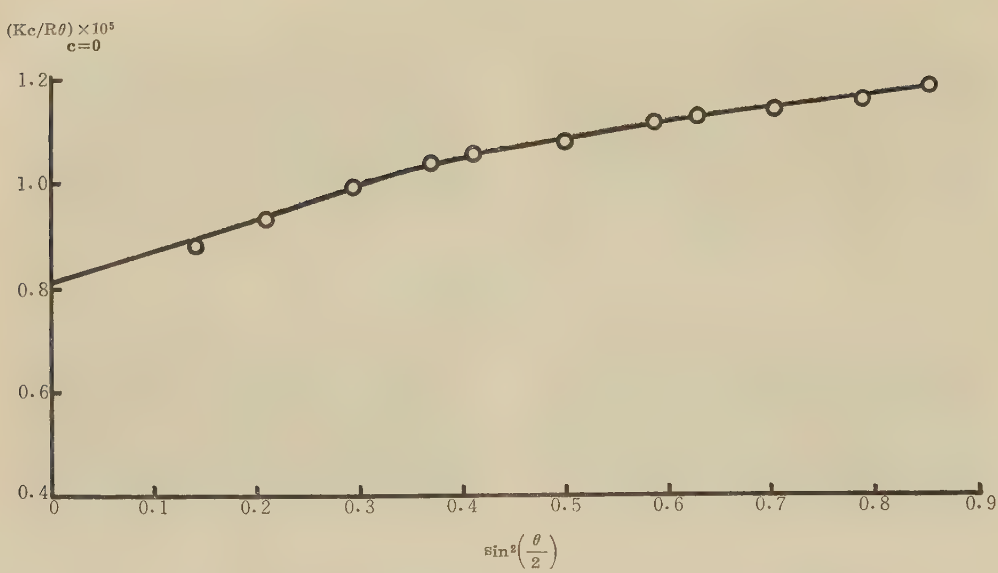


Fig. 13. Reduced intensity of light-scattering as an angular envelope of protein A
Condition: ionic strength 1.25 $KCl-K_2HPO_4-KH_2PO_4$, pH 7.05

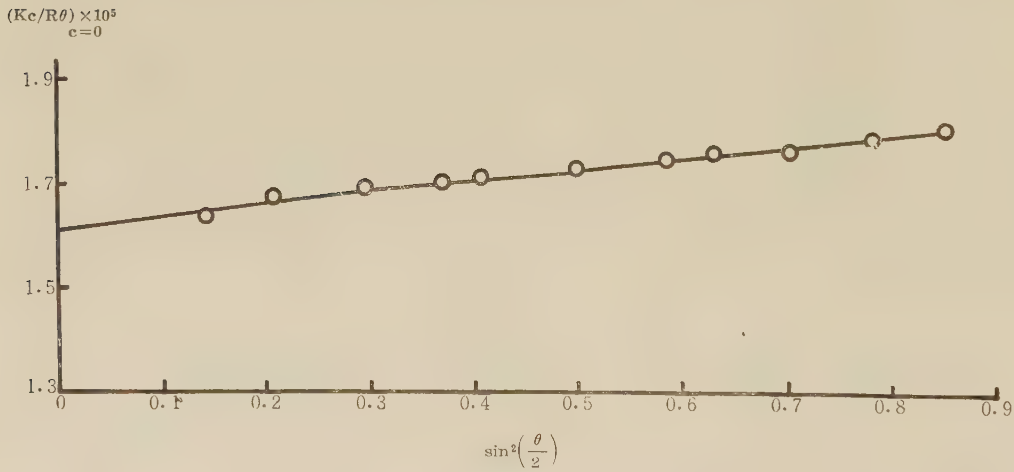


Fig. 14. Reduced intensity of light-scattering as an angular envelope of protein B
Conditions: ionic strength 1.25 $KCl-K_2HPO_4-KH_2PO_4$, pH 7.05

1,100 Å) of protein A are about twice as large as these of protein B (molecular weight 62,000, rod 720 Å, coil 510 Å). The values of protein B are somewhat larger than these of fish tropomyosin (molecular weight 55,000, rod 520 Å, coil 370 Å) and of rabbit tropomysin (molecular weight 53,000, rod 490 Å, coil 350 Å).

The molecular weight of rabbit tropomyosin obtained by the light-scattering method agreed well with the results obtained by the osmotic pressure method by

Table 2. Molecular weight and length of protein A, B, rabbit and fish (haddock) tropomyosins

Protein	$(Kc/R_\theta)_{c=0}\times10^5$	M	r_g	Length (Å)
Protein A	0.802	125,000	449	1,560 (rod)
				1,100 (coil)
Protein B	1.610	62,000	208	720 (rod)
				510 (coil)
Rabbit tropomyosin	1.890	53,000	142	490 (rod)
				350 (coil)
Fish tropomyosin	1.810	55,000	151	520 (rod)
				370 (coil)

Solution, ionic strength 1.25 (1.20 KCl, 0.05 K_2HPO_4-KH_2PO_4); n_0=1.340; r_g, radius of gyration; M, molecular weight.

Tsao et al.[6] (53,000 in 6.7 M urea) and by Doty and Sanders by the light-scattering method cited in Bailey[41] (53,000~55,000 at ionic strength 1.01 and 100,000 at ionic strength 0.1)

IV. Amino acid Composition of Proteins A and B

In the usual procedure of determination of the amino acid composition of protein, certain amino acids, such as serine and threonine, are susceptible to decomposition[42]. Beside these amino acids, glutamic and aspartic acid also suffer loss to some extent[43-44]. The destruction rates of these amino acids depend not only upon the duration of hydrolysis, but also upon the kind of protein. On the other hand, compounds in which the carboxyl group of isoleucine and valine are coupled in peptide linkage are not easily hydrolysed[43-46].

Therefore, to overcome these difficulties, two different length-periods of hydrolysis have been adopted in the following determinations.

1) Methods

The dried protein was suspended with about 200 volumes of three times glass-distilled HCl in a pyrex tube, and the solution was frozen in a bath of solid carbon dioxide and ethanol. After having been evacuated to about 2 mm pressure, the tube was sealed. Hydrolysis was performed in an oven at 105±1°C for 22 or 72 hours. After hydrolysis of the protein during the appropriate hours, the pale yellow hydrolysate was filtered on a glass filter and excess HCl was removed by repeated drying in vacuo. The residue was dissolved in water and made up to a desired volume. Total nitrogen of the hydrolysate was determined by the micro-Kjeldahl method and the nitrogen value of 18.1% was used to calculate the weight of protein.

The chromatography of the hydrolysate from 2.5~3.0 mg protein was performed on Dowex 50-X 8 column by the method of Moore and Stein[47], except for no addition of detergent to all the buffers employed: a 0.9×100 cm column

was used for acidic and neutral amino acids and a 0.9×15 cm column for basic amino acids and amide nitrogen. Eluted fractions were analysed by the ninhydrin procedure according to Moore and Stein with slight modifications[48]. To determine tyrosine and phenylalanine, the fraction of tyrosine plus phenylalanine was obtained by 0.9×15 cm column chromatography while the tyrosine value was directly measured on the hydrolysate by the method of Ceriotti and Spandrio[49]. Since the proline content of the proteins was low, it was independently measured by the method Chinard[50] on the fraction of glutamic acid and its adjacent in 15~18 mg protein hydrolysate. After the oxidation of protein with performic acid according to the method of Schram et al.[51], cystine and cyteine were determined as cysteic acid on 25~40 ml fraction of the long column with 15~18 mg protein hydrolysate. Amide nitrogen was determined independently by the method of Bailey[52] in order to check the values calculated from the chromatographic results.

2) Results

The elution curves of 22- and 72-hour-hydrolysates on the long column are given in Figs. 15 and 16 for protein A and in Figs. 17 and 18 for protein B.

In the case of the 22-hour-hydrolysates of both these proteins, unknown peaks were recognized before and after the valine fraction and in front of the methionine. In 72-hour-hydrolysates several unknown peaks were revealed only in front of methionine. Since the amounts of valine and isoleucine in 72-hour-hydrolysate are higher than those of 22-hour, the peaks before and after valine may be due to some peptides which contain valine and/or isoleucine. On the other hand, the peaks eluted before methionine seem to be artifacts due to the change of the buffer.

The elution curves of 22- and 72-hour-hydrolysates in the short column are shown in Figs. 19, 20 (protein A) and Figs. 21, 22 (protein B). After ammonia peaks, several other peaks were revealed, which may be due to the artifacts of the change of pH 6.5 buffer.

The analytical results obtained from three pairs of samples of 22- and 72-hour-hydrolysates of proteins A and B are presented in Table 3. A comparison of the analytical values of each hydrolysate indicates that certain amino acids, such as valine and isoleucine, and ammonia are extremely increased, but serine and threonine are decreased by increase in the length of hydrolysis. The decomposition of aspartic acid, glutamic acid, lysine and methionine, which has been reported by several previous investigators[18,43-44], was not observed in the present analysis.

The values for threonine and serine were obtained by extrapolating the results of 22- and 72-hour-hydrolyses to zero time, assuming that the losses of these amino acids occurred linearly with the time of hydrolysis. As the value of ammonia obtained by chromatography fluctuated greatly, the value gained independently by the method of Bailey[52] was adopted as valid. For the values of isoleucine and valine were taken those of 72-hour-hydrolysis. The average values of 22- and 72-hydrolysates were adopted for the amounts of other amino acids. The amino acid compositions of proteins A and B thus obtained are summarized in Tables 4 and 5, respectively. The results of the present amino

Table 3. Effect of hydrolysis time on the amounts of amino acids eluted from proteins A and B
(Nitrogen as per cent of total protein nitrogen)

Amino acid	Protein A		Protein B	
	22 hour	72 hour	22 hour	72 hour
Glycine	2.01±0.05	2.03±0.03	1.84±0.07	1.83±0.02
Alanine	6.89±0.03	6.88±0.01	7.24±0.02	7.26±0.05
Valine	2.76±0.10	2.82±0.04	2.31±0.06	2.42±0.03
Leucine	7.45±0.11	7.35±0.15	6.68±0.17	6.76±0.09
Isoleucine	2.32±0.03	2.70±0.04	1.82±0.01	2.16±0.02
Proline	0.25±0.05	0.25±0.03	0.16±0.03	0.18±0.02
Phenylalanine	0.77±0.20	0.73±0.18	0.92±0.25	0.95±0.20
Methionine	0.92±0.06	0.98±0.05	1.69±0.04	1.61±0.05
Arginine	23.98±0.11	23.92±0.05	20.02±0.13	20.03±0.13
Histidine	2.94±0.07	2.95±0.08	2.27±0.06	2.23±0.09
Lysine	9.78±0.10	9.74±0.05	12.20±0.11	12.26±0.09
Aspartic acid	7.80±0.04	7.78±0.05	7.97±0.13	7.81±0.11
Glutamic acid	13.69±0.28	13.73±0.30	16.68±0.28	16.58±0.32
Serine	3.76±0.02	3.56±0.03	3.54±0.05	3.22±0.04
Threonine	2.99±0.07	2.81±0.05	1.70±0.03	1.65±0.04
Tyrosine	0.81±0.04	0.78±0.02	0.49±0.05	0.49±0.03
Amide Nitrogen	8.10±0.41	8.40±0.29	7.66±0.35	8.02±0.46

acid analysis seem to be satisfactory, as the recovery of amino acid nitrogen per total nitrogen was 98.03 and 95.72% respectively in proteins A and B. On the basis of the results exhibited in Tables 4 and 5, the side chain properties of proteins A and B are given as in Table 6. The amino acid patterns of these two proteins are rather unique: the amounts of glutamic acid, aspartic acid, alanine, leucine, arginine and lysine are remarkably high, but the amounts of histidine, tyrosine, phenylalanine, half cystine and proline are low. As shown in Table 6, the amounts of free acidic, basic and polar side chain groups are very high.

Some differences in these two proteins are obvious in respect to the contents of alanine, leucine, methionine, threonine, glutamic acid, lysine, arginine and amide nitrogen. Especially amongst the basic amino acids, the ratio of the contents of lysine and arginine of protein A replaces that of protein B and the content of glutamic acid of protein A is lower than that of protein B. The sum of free acidic and basic groups and net negative charge at pH 8 are also distinguishably different from each other.

V. Terminal Residues in Proteins A and B

To demonstrate difference in the chemical structures of proteins A and B, the terminal residues of these proteins were examined.

1) Methods

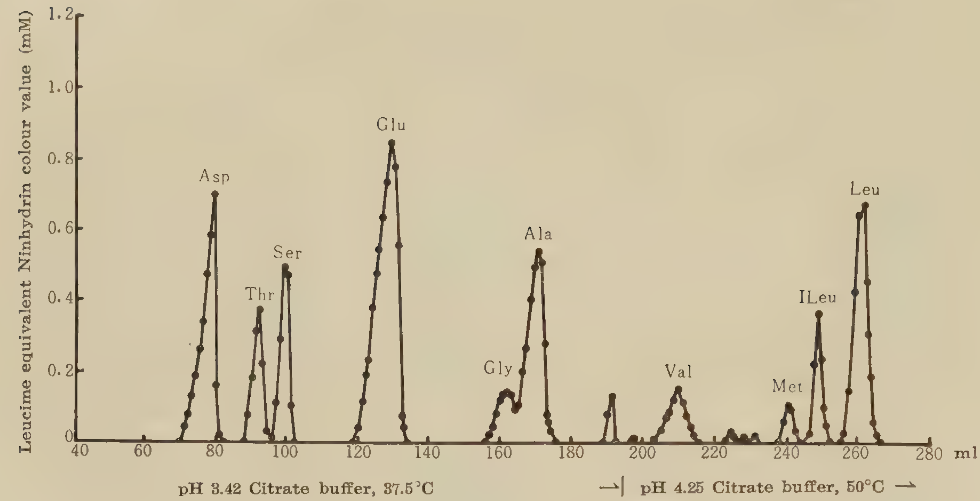


Fig. 15. Elution curve for a 22-hour-hydrolysate of protein A on a 0.9×100 cm column Dowex-50 (X-8), 3.02 mg protein

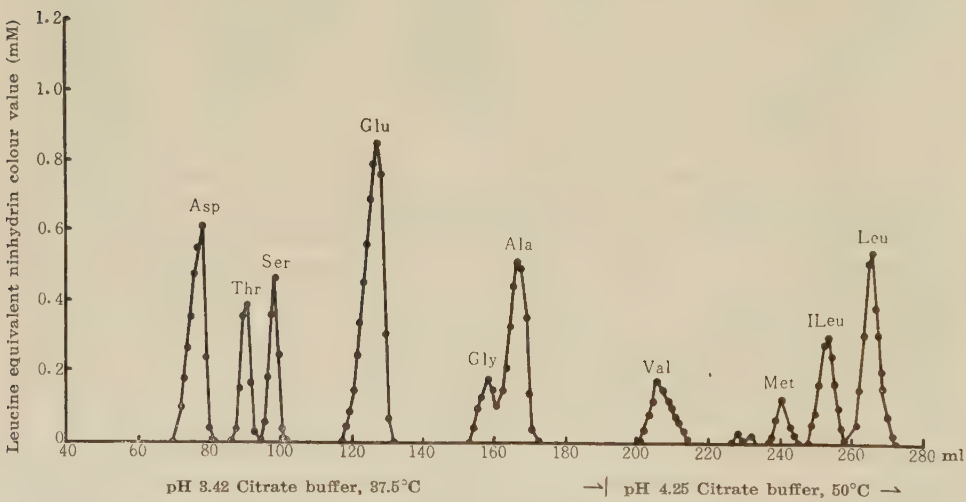


Fig. 16. Elution curve for a 72-hour-hydrolystae of protein A on a 0.9×100 cm column Dowex-50 (X-8), 2.90 mg protein

N-terminal assay: The dinitrofluorobenzene (DNFB) method of Sanger[53] was applied for the examination and estimation of N-terminal residue in these two proteins. The dinitrophenyl-protein (DNP-protein), which was prepared under the conditions recommended by Sanger, was hydrolysed in a sealed glass tube by 6 N HCl at 100°C during appropriate hours. The hydrolysate was extracted with ether, and separated into ether soluble and water soluble fractions.

After sublimation of dinitrophenol, dinitrophenyl-amino acids (DNP-amino

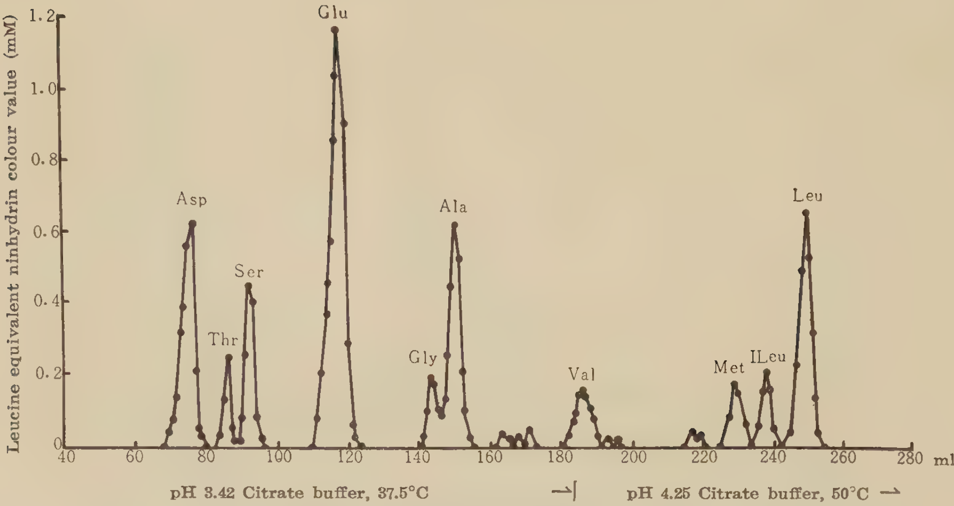


Fig. 17. Elution curve for a 22-hour-hydrolystae of protein B on a 0.9×100 cm column Dowex-50 (X-8), 2.92 mg protein

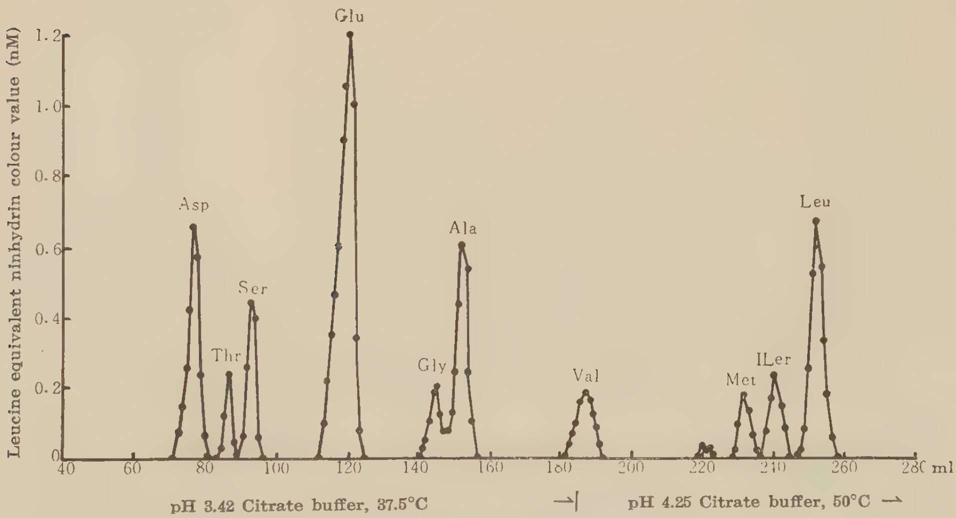


Fig. 18. Elution curve for a 72-hour-hydrolystae of protein B on a 0.9×100 cm column Dowex-50 (X-8), 2.94 mg protein

acids) in the ether soluble fraction were chromatographed on No. 51 Toyo filter paper by the method of Levy[54-55], and eluted with 1% $NaHCO_3$. Their amounts were measured colorimetrically at 360 mμ, adopting the molar extinction coefficient of 1.70×10^4. DNP-amino acids in the water soluble fraction were absorbed in a talc column[56]. After elution with N HCl-ethanol (1 : 4), it was chromatographed in tert-amyl alcohol[55].

For the inspection of the terminal cysteyl residue[57], the protein oxidized

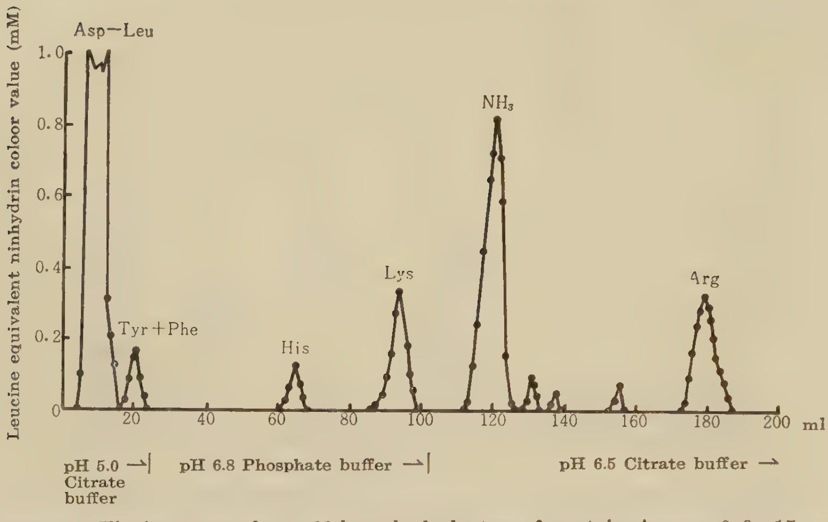


Fig. 19. Elution curve fro a 22-hour-hydrolystae of protein A on a 0.9×15 cm column Dowex-50 (X-8), 3.02 mg protein

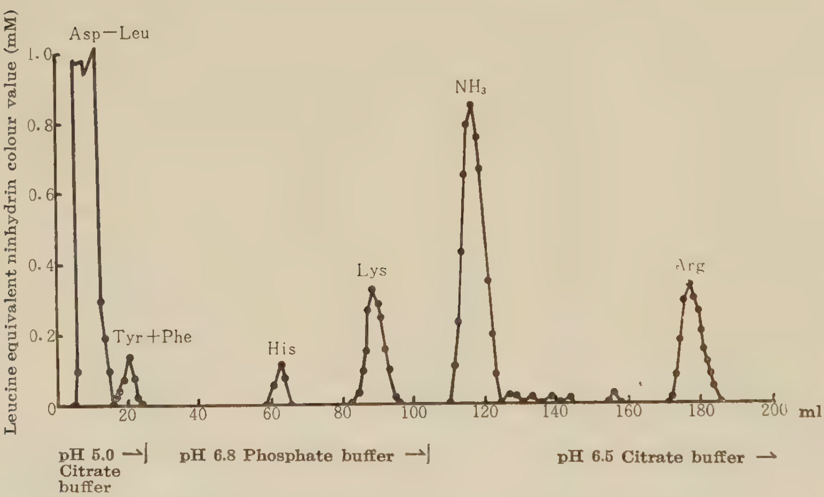


Fig. 20. Elution curve for a 72-hour-hydrolystae of protein A on a 0.9×15 cm column Dowex-50 (X-8), 2.90 mg protein

by the method of Schram et al.[51)] was treated with DNFB: DNP-amino acid in the water soluble fraction was examined by the same method as described above. In this assay, the amounts of the original protein were calculated from the amide nitrogen in the DNP-protein.

C-terminal assay: C-terminal residue was measured by the method of Ohno and Akabori[58-61)], use being made of about 50 mg of the protein which was previously dried over P_2O_5 at 110°C for 12 hours. The protein was hydrazinolysed with anhydrous hydrazine at 100°C for an appropriate time, and excess

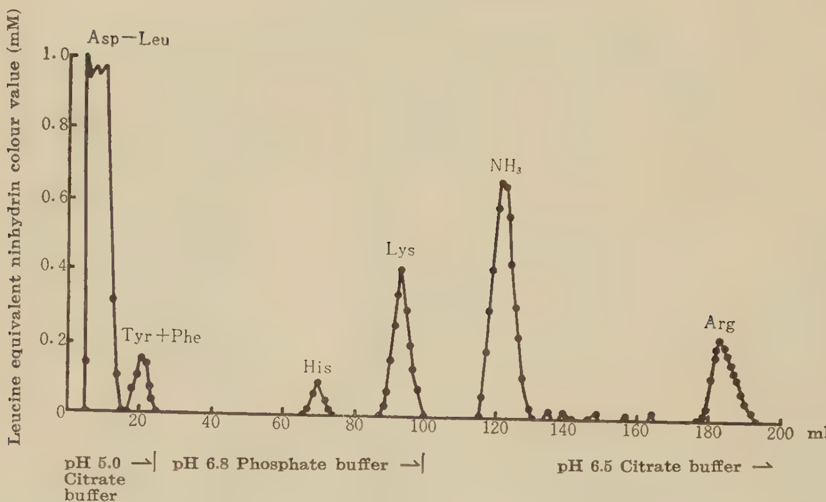


Fig. 21. Elution curve for a 22-hour-hydrolysate of protein B on a 0.9×15 cm column Dowex-50 (X-8), 2.92 mg protein

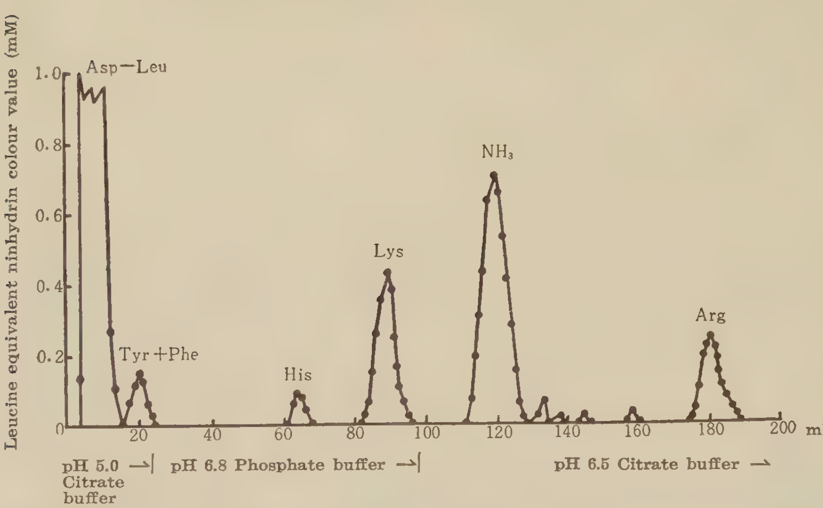


Fig. 22. Elution curve for a 72-hour-hydrolysate of protein B on a 0.9×15 cm column Dowex-50 (X-8), 2.94 mg protein

hydrazine was removed in vacuo over conc. H_2SO_4. Amino acid hydrazide was removed by twice repetition of treatment with isovaleraldehyde. After removal of isovaleraldehyde with ethyl acetate, the water soluble fraction was treated with DNFB. The mixture was acidified with 2 N HCl, and extracted with ethyl acetate. The ethyl acetate fraction was washed with 2% $NaHCO_3$*. The $NaHCO_3$

* Most of DNP-amino acids and a part of isovaleral derivatives of DNP-aspartic and glutamic monohydrazides are transferred into $NaHCO_3$ fraction, while all of di-DNP-tyrosine and a part of DNP-tryptophan remain in the ethyl acetate layer. Di-DNP-tyrosine in this acetate fraction could not be detected in proteins A and B. Tryptophan was not included among these proteins.

Table 4. Amino acid composition of protein A

Amino acid	Amino acid N per total N (%)	Amino acid weight per 100 g protein (%)	Amino acid residue weight per 100 g protein (%)	Amino acid moles per 10^5 g protein
Glycine	2.02	1.95	1.48	26
Alanine	6.89	7.94	6.34	89
Valine	2.82	4.27	3.61	37
Leucine	7.40	12.54	10.82	96
Isoleucine	2.70	4.58	3.95	35
Proline	0.25	0.37	0.31	3
Phenylalanine	0.75	1.60	1.43	10
Half Cystine	0.37	0.57	0.48	5
Methionine	0.95	1.83	1.61	12
Tryptophan	—	—	—	—
Arginine	23.95	13.50	12.11	78
Histidine	2.95	1.97	1.74	13
Lysine	9.76	9.21	8.07	63
Aspartic acid	7.79	13.40	11.59	101
Glutamic acid	13.71	26.04	22.86	177
Serine	3.86	5.26	4.36	50
Threonine	3.08	4.75	4.03	40
Tyrosine	0.80	1.87	1.69	10
Amide Nitrogen	7.98	(1.45)	—	(104)
Total	98.03	111.65	96.48	845

( ), omitted from the total value

Table 5. Amino acid composition of protein B

Amino acid	Amino acid N per total N (%)	Amino acid weight per 100 g protein (%)	Amino acid residue weight per 100 g protein (%)	Amino acid moles per 10^5 g protein
Glycine	1.84	1.78	1.36	24
Alanine	7.25	8.34	6.65	94
Valine	2.42	3.66	3.10	31
Leucine	6.72	11.37	9.81	87
Isoleucine	2.16	3.66	3.16	28
Proline	0.17	0.25	0.22	2
Phenylalanine	0.94	2.00	1.78	12
Half Cystine	0.22	0.34	0.29	3
Methionine	1.65	3.17	2.79	21
Tryptophan	—	—	—	—
Arginine	20.03	11.29	10.13	65
Histidine	2.25	1.50	1.33	10
Lysine	12.23	11.54	10.12	79
Aspartic acid	7.84	13.42	11.61	101
Glutamic acid	16.63	31.60	27.74	215
Serine	3.68	5.10	4.15	48
Threonine	1.79	2.76	2.34	23
Tyrosine	0.49	1.15	1.04	6
Amide Nitrogen	7.42	(1.34)	—	(96)
Total	95.72	112.38	97.15	849

( ), omitted from the total value

Table 6. Side chain properties of proteins A and B

Group	Protein A (%)	Protein B (%)
Free acid group	20.6) 38.8	26.6) 44.1
Basic group	18.2	18.1
Hydroxy group	11.8	9.2
Amide group	12.3	11.3
Polar group	63.5	64.8
Nonpolar group	36.9	35.2
Net negative charge at pH 8	33	76

Table 7. DNP-amino acids as N-terminals of proteins A and B*

Protein A				Protein B			
Amino acid	Hydrolysis time (hour)			Amino acid	Hydrolysis time (hour)		
	4	8	12		4	8	12
Leu or ILeu	0.0_6	0.0_7	0.0_4	Thr	0.1_2	0.1_1	0.0_9
Val	0.1_0	0.1_0	0.0_6				
Ala	0.0_2	0.0_3	0.0_2				

* All amounts are expressed as moles per mole of protein

fraction was acidified and extracted with ether. C-terminal DNP-amino acid in the ether fraction was estimated by the method described above in the paragraph on N-terminal assay. Further to identify the DNP-amino acids, the eluted solution of DNP-amino acids was hydrolysed with HCl for 4 hours, and was rechromatographed.

In these assays, 125,000 and 62,000 were adopted as the molecular weights of proteins A and B, respectively.

2) Results

N-terminal residue: In the ether soluble fraction of protein A, DNP-alanine, -valine and -leucine or -isoleucine were detected, and in the ether soluble fraction of protein B, DNP-threonine was detected, as shown in Table 7. The amount of each DNP-amino acid, however, was not more than 0.1 mole per mole of protein. Therefore, these DNP-amino acids seem to arise from impurities of the protein preparations. In the water soluble fraction, only ε-DNP-lysine was detected in these two proteins and also in the performic acid-oxidized proteins A and B. Therefore, it may be concluded that by the DNFB method no N-terminal residue can be detected in the proteins A and B.

C-terminal residue: Certain amounts of DNP-serine, and traces of DNP-valine, -alanine and -isoleucine or -leucine were detected in protein A. DNP-isoleucine or -leucine, and a trace of DNP-threonine were dected in protein B. To identify DNP-isoleucine or -leucine, the eluted spot was hydrolysed with

Table 8. DNP-amino acids as C-terminals of proteins A and B*

Protein A			Protein B		
Amino acid	Hydrazinolysis time (hour)		Amino acid	Hydrazinolysis time (hour)	
	5	10		5	10
Ser	0.6_7	0.5_9	ILeu	0.6_8	0.5_7
Leu or ILeu	trace	trace	Thr	0.1_0	0.0_4
Val	0.0_5	0.0_8	Glu or Asp	trace	trace
Ala	trace	trace			

* All amounts are expressed as moles per mole of protein

conc. ammonia at 100°C for 2 hours[62], and only isoleucine was detected by chromatography in tert-amyl alcohol at pH 6.0[55].

The amounts of the DNP-amino acids are given in Table 8. It is obvious from this table that protein A has one C-terminal reisdue, viz., serine, and protein B has one isoleucine as C-terminal residue. Other amino acids may be due to impurities.

Discussion

All the tropomyosins, whose amino acid compositions have been presented, show several specific patterns in their respective compositions as given in Table 9, in which the amino acids of vertebrata and invertebrata tropomyosins are tabulated in regular sequence according to their amounts. They show high contents of glutamic acid, aspartic acid, alanine, leucine, lysine and arginine, but have small amounts of histidine, methionine, proline, phenylalanine, tyrosine and half cystine. They have no tryptophan. Beside these characteristics of amino acid compositions, they all show a particularly high content of polar side chain (62~66%) and of ionic side group (35~47%), as reported in Table 10. The amino acid compositions and the side chain properties of proteins A and B, listed in Table 6, also show these specific patterns common to all tropomyosins.

In spite of these common properties of the tropomyosins, there are several differences in their amino acid compositions and side chain properties. It is sight obvious from Table 9 that in vertebrate tropomyosin the content of lysine is much higher that that of aspartic acid, but, on the contrary, in invertebrate tropomyosin the content of lysine is lower than that of aspartic acid. It is also interesting to note that in the vertebrate, arthropod and some of the mollusc tropomyosins the content of lysine is higher than that of arginine, but in other mollusc and annelide tropomyosins, the lysine content is lower than the arginine content. As shown in Table 10, tropomyosins can be divided into two groups, the one having comparatively higher content (43~47%) and the other having lower content (35~37%) of ionic side groups.

Yoshimura[22] and Bailey[23,27] have already reported some specificity in the arginine and lysine contents of mollusc tropomyosins. Not only in mollusc tropo-

Table 9. Amino acid compo
(Amino acid moles p

Vertebrate								Arthropod					
Rabbit skeletal (8)		Frog (21)		Carp (21)		Haddock (18)		Homarus (29)		Protein B		Squid mantle (22)	
1. Glu	211	1. Glu	201	1. Glu	201	1. Glu	237	1. Glu	200	1. Glu	215	1. Glu	212
2. Lys	110	2. Lys	112	2. Lys	113	2. Lys	114	2. Asp	101	2. Asp	101	2. Asp	99.
3. Ala	110	3. Ala	106	3. Leu	107	3. Ala	101	3. Ala	90	3. Ala	94	3. Ala	95
4. Leu	95	4. Leu	95	4. Ala	106.5	4. Leu	101	4. Leu	88	4. Leu	87	4. Leu	95
5. Asp	89	5. Asp	91	5. Asp	96	5. Asp	85	5. Lys	78	5. Lys	79	5. Lys	85
6. Arg	42	6. Arg	42	6. Arg	41	6. Ser	40	6. Arg	63	6. Arg	65	6. Arg	69
7. Ser	40	7. Ser	34	7. Val	37	7. Arg	38	7. Ser	43	7. Ser	48	7. Ser	55
8. Val	38	8. Val	32	8. Ser	33	8. Thr	34	8. Val	33	8. Val	31	8. Thr	33
9. ILeu	29	9. ILeu	29	9. ILeu	30	9. ILeu	32	9. Thr	27	9. ILeu	28	9. Gly	28
10. Thr	28	10. Thr	25	10. Thr	24	10. Val	29	10. Met	22	10. Gly	24	10. Val	25
11. Met	16	11. Met	25	11. Met	23	11. Tyr	21	11. Gly	14	11. Thr	23	11. ILeu	20
12. Try	15	12. Tyr	18	12. Tyr	18.5	12. Met	18	12. ILeu	12	12. Met	21	12. Met	17
13. Gly	12.5	13. Gly	14	13. Gly	18	13. Gly	12	13. Tyr	12	13. Phe	12	13. His	12
14. Cys/2	6.5	14. His	7	14. His	6	14. Cys/2	6.5	14. Phe	11	14. His	10	14. Phe	8
15. His	5.5	15. Cys/2	6	15. Phe	6	15. Phe	6	15. His	3	15. Tyr	6	15. Thr	8
16. Phe	3.5	16. Phe	4	16. Cys/2	5	16. His	3.5	16. Pro	3	16. Cys/2	3	16. Cys/2	6
		17. Pro	3	17. Pro	3					17. Pro	2		
Total	851	Total	844	Total	868	Total	878	Total	800	Total	849	Total	867.

ions of various tropomyosins
10^5 g of tropomyosin)

Mollusc												Annelid	
Loligo mantle (29)		Verus foot, adductor tropomyosin B (29)		Protein A		Verus foot, adductor tropomyosin A (29)		Pinna adductor (27)		Octopus (27)		Arenicola (29)	
1. Glu	195	1. Glu	205	1. Glu	177	1. Glu	169	1. Glu	173	1. Glu	160	1. Glu	156
2. Asp	103	2. Asp	110	2. Asp	101	2. Asp	114	2. Asp	111	2. Leu	104	2. Asp	120
3. Ala	93	3. Ala	101	3. Leu	96	3. Ala	108	3. Leu	103	3. Asp	91	3. Leu	106
4. Leu	86	4. Leu	88	4. Ala	89	4. Leu	106	4. Ala	102	4. Ala	78.5	4. Ala	87
5. Lys	78	5. Lys	78	5. Arg	78	5. Arg	81	5. Arg	79	5. Arg	70	5. Arg	85
6. Arg	63	6. Arg	67	6. Lys	63	6. Lys	59	6. Lys	64.5	6. Lys	60	6. Lys	56
7. Ser	45	7. Ser	43	7. Ser	50	7. Ser	39	7. Ser	49	7. Ser	52	7. Ser	44
8. Thr	38	8. Val	40	8. Thr	40	8. Thr	36	8. Val	35.5	8. Thr	37	8. Thr	33
9. ILeu	25	9. Thr	35	9. Val	37	9. Val	28	9. ILeu	28	9. ILeu	34	9. Val	30
10. Val	24	10. ILeu	27	10. ILeu	35	10. ILeu	22	10. Thr	22	10. Val	32	10. ILeu	25
11. Gly	17	11. Gly	20	11. Gly	26	11. Thr	18	11. Met	15.5	11. Gly	16	11. Gly	25
12. Met	12	12. Tyr	20	12. His	13	12. Gly	15	12. Tyr	14	12. Met	13	12. Met	13
13. Phe	12	13. Met	8	13. Met	12	13. Met	11	13. Gly	12	13. His	11	13. Tyr	10
14. Tyr	10	14. Phe	6	14. Tyr	10	14. Phe	6	14. Phe	8.5	14. Tyr	8	14. Phe	6
15. Pro	4	15. His	3	15. Phe	10	15. His	4	15. His	4.5	15. Phe	6.5	15. His	6
16. His	3	16. Pro	2	16. Cys/2	5	16. Pro	1.5	16. Cys/2	2.5			16. Pro	4
				17. Pro	3								
Total	803	Total	853	Total	845	Total	817.5	Total	824	Total	779	Total	806

myosins but also in several animals' tropomyosins, Kominz and Laki[25,29] have observed that the number of net negative charge and the ratio of lysine to arginine decrease regularly as the phyla of animal origin have a lower position from the view-point of evolutionary theory. They have classified tropomyosins into the two groups: tropomyosins A and B. Tropomyosin A salts out at 26~35% ammonium sulphate saturation; its net negative charge is 25~35 at pH 8, and its content of arginine is higher that that of lysine. Tropomyosin B salts out at 41~55% saturation; its net negative charge is about 80, and its content of lysine is higher than that of arginine. On the other hand, Bailey[27] has divided tropomyosins into water-insoluble and -soluble. These two tropomyosins perhaps correspond to the groups A and B of Kominz and Laki, respectively. It is apparent

Table 10. Side chain properties of various tropomyosins

Group	Vertebrate			
	Rabbit skeletal (8) (%)	Frog (21) (%)	Carp (21) (%)	Haddock (18) (%)
Free acid group	27.8) 46.3	28.1) 47.2	27.0) 45.5	29.7) 47.7
Basic group	18.5	19.1	18.5	18.0
Hydroxy group	9.8	9.1	8.8	10.8
Amide group	7.4	6.5	7.2	7.0
Polar group	64.3	63.5	62.1	66.2
Nonpolar group	35.7	36.5	38.9	33.8

Group	Arthropod	Mollusc		
	Homarus (29) (%)	Squid mantle (22) (%)	Loligo mantle (29) (%)	Verus foot, adductor tropomyosin B (29) (%)
Free acid group	26.4) 44.4	25.0) 44.1	26.2) 44.1	27.1) 44.5
Basic group	18.0	19.1	17.9	17.4
Hydroxy group	10.2	11.0	11.6	11.5
Amide group	11.2	10.9	10.6	9.8
Polar group	65.8	66.6	66.5	65.9
Nonpolar group	34.2	33.4	33.5	34.1

Group	Mollusc			Annelid
	Verus foot, adductor tropomyosin A (29) (%)	Pinna adductor (27) (%)	Octopus foot (27) (%)	Arenicola (29) (%)
Free acid group	21.2) 38.8	19.3) 36.3	18.4) 35.3	20.2) 38.4
Basic group	17.6	17.0	16.9	18.2
Hydroxy group	11.4	10.3	12.4	10.8
Amide group	13.5	13.3	10.6	14.0
Polar group	63.7	63.1	63.6	63.3
Nonpolar group	36.3	36.9	36.4	36.7

from the results presented (Figs. 7, 8, Tables 4, 5, 6 and 9) that the salting-out range, the amounts of negative charge and the ratio of lysine to arginine of protein A correspond to tropomyosin A of Kominz and Laki or to the water-insoluble tropomyosin of Bailey and that all properties of protein B to the tropomyosin B of Kominz and Laki or to the water-soluble tropomyosin of Bailey.

Squid tropomyosins have already been prepared by Yoshimura[22] from the mantle of the animal used in this report (*Ommarstrephes sloani pacificus* STEENSTRUP), by Tsao et al.[12] from *Sepia esculenta*, and by Kominz and Laki[29] from *Loligo pealeii*. All these tropomyosins belong to tropomyosin B group or water-soluble tropomyosin group in respect to their solubility, salting-out range, amino acid composition and net negative charge. This seems to be the first time that two types of tropomyosin have been isolated from the muscle of squid mantle, cephalopoda, as described in this report. In the isolation of these proteins, the previous investigators have applied Bailey's procedure which was originally adopted for rabbit skeletal muscle[2]. In the procedure, protein A would be denatured by the treatment of acid and removed by fractionation with 41~55% ammonium sulphate saturation. On the other hand, Bailey's method[24,27] for isolation of water-insoluble tropomyosins from adductor muscle and octopus foot muscle might be unsuitable for the squid mantle, as the proteins A and B have similar solubility against dilute KCl solution.

The protein B has the same salting-out range, C-terminal amino acid, and amino acid composition pattern, as those of Yoshimura's tropomyosin[22]. Differences are observed in nitrogen contents, and in solubility in KCl of these two proteins. However, these two tropomyosins may be identical, because these differences are not substantial. The difference in solubility might be due to its change by subjection to high (about 9.0) or low (below 4.5) pH. The low nitrogen content in Yoshimura's tropomyosin might reflect its incomplete dryness.

N-terminal residue could not be detected in the two proteins A and B by the usual DNFB method, but one serine residue and one isoleucine residue respectively were detected as C-terminal by the use of anhydrous hydrazine. Similar results have already been observed in other tropomyosins: no N-terminal residue has been detected in any of the tropomyosins isolated at present (the adductor muscle of pinna[27], the foot muscle of octopus[27], rabbit skeletal muscle[5], pig heart[14], duck gizzard[14], frog[19], fish[19] and the body muscle of prawn[14]). On the other hand, Locker[7] established the existence of one isoleucine as C-terminal residue in rabbit skeletal tropomyosin by the use of carboxypeptidase and of anhydrous hydrazine. He has found isoleucine-serine-threonine-methionine-isoleucine-alanine- as the C-terminal sequence. The present author has previously found one C-terminal isoleucine residue in the tropomyosins isolated from frog and fish skeletal muscle by the use of anhydrous hydrazine[19]. By the use of the method of carboxypeptidase A, Kominz and Lake have reported two C-terminal residues, viz., isoleucine and serine in rabbit skeletal and bladder muscle, asparagine and leucine in uterus muscle tropomyosin[10], isoleucine and probably serine in frog tropomyosin[21]. However, these descrepancies in the number of C-terminal residue may be attributable to the inherent difficulty of application of the carboxypeptidase method. To identify C-terminal residue by this method, it is necessary

to check up time sequence and the effect of the ratio of substrate to enzyme. It is interesting that all these tropomyosins have no detectable N-terminal amino acid, in connection with the recent observation of the existence of masked N-terminal amino acid in tobacco mosaic virus protein[63].

Not only do the proteins A and B differ very much from each other in their molecular weights and lengths, but also they differ from the tropomyosins of rabbit and fish which have similar molecular sizes nad shapes. The molecular weights of the tropomyosins vary greatly from one another; rabbit 53,000, duck gizzard 150,000[12], pig cardiac 89,000[12], fish 55,000, prawn 77,0000[12], sepia 68,000[12], squid B 62,000, squid A 125,000 and pinna 137,000[30]. However, all these tropomyosin molecules are highly asymmetric and their molecular weights increaes generally in proportion to the increase in molecular length or axial ratio. Furthermore all tropomyosins show several common patterns in their amino acid compositions. These observations may suggest that tropomyosins are similar in their molecular pattern and that the difference in their size is attributable to the different lengths along the long axis.

The close relationship between paramyosin filament and tropomyosin has recently been confirmed by many workers[24,28,64-66], especially in respect to the periodical pattern of paramyosin filament in x-ray diffraction and the molecular length of water-insoluble tropomyosin. It is now generally accepted that paramyosin is identical to water-insoluble tropomyosin or that it is composed mainly of water-insoluble tropomyosin. Therefore the paramyosin filament may be contained in squid mantle, since tropomyosin A can be isolated from the mantle.

Note Added in Proof

Two kinds of tropomyosins have been isolated in homogeneous, crystalline form from the adductor muscle of *Pecten maximus* by Bailey and Rüegg. Their solubility, electrophoretic characteristics and amino acid composition have been elucidated. (cf. Bailey, K. and Rüegg, J. C. (1960). Further chemical studies on the tropomyosins of lamellibranch muscle with special reference to *Pecten maximus*. *Biochim. et Biophys. Acta* 38, 239.)

Summary

1. Two kinds of crystalline proteins in the tropomyosin fraction have been isolated from the dehydrated muscle of squid mantle by the purification procedure with ammonium sulphate fractionation.

2. The crystalline protein A salted out in the range of 21~39% ammonium sulphate saturation and the crystalline protein B in 40~54% saturation.

3. The electrophoretic mobilities of these proteins were measured in KCl-phosphate buffer of pH 7.15 and ionic strength 0.45 at 0°C. The mobility of the protein A (desc. 1.65 and asc. 1.79×10^{-5} cm^2/volt/sec) was lower than that of the protein B (desc. 2.10 and asc. 2.25×10^{5} cm^2/volt/sec).

4. These proteins showed a characteristic tropomyosin pattern in respect

to amino acid contents and especially in side chain properties of amino acids, though they obviously differed from each other in the sum of free acidic and basic groups, net negative charge and the ratio of contents of lysine and arginine.

5. The molecular weight of proteins A and B were determined respectively to be 125,000 and 62,000 by the technique of light-scattering. These values are higher than that of rabbit, 53,000 and of fish (haddock) tropomyosins, 55,000. The molecular lengths of proteins A (rod 1,560 Å, coil 1,100 Å) was higher than those of protein B (rod 720 Å, coil 510 Å) and also those of rabbit (rod 490 Å, coil 350 Å) and fish tropomyosins (rod 520 Å, coil 370 Å).

6. No N-terminal residue was detected in these two proteins by the use of DNFB method, but one C-terminal serine was obtained in the protein A and one C-terminal isoleucine in the protein B by the use of anhydrous hydrazine.

7. From the salting-out ranges and the patterns of amino acid composition, it was concluded that the protein A belongs to water-insoluble or A group and the protein B to water-soluble or B group.

References

1) Bailey, K. (1946). *Nature* **157**, 368.
2) ——— K. (1948). *Biochem. J.* **43**, 271.
3) ———, Gutfreund, H, & Ogston, A. G. (1948). *Ibid.* **43**, 279.
4) Astbury, W. T., Reed, R. & Spark, L. C. (1948). *Ibid.* **43**, 282.
5) Bailey, K. (1951). *Ibid.* **49**, 23.
6) Tsao, T. C., Bailey, K. & Adair, G. S. (1951). *Ibid.* **49**, 27.
7) Locker, R. H. (1954). *Biochim. et Biophys. Acta* **14**, 533.
8) Kominz, D. R., Hough, A., Symonds, P. & Laki, K. (1954). *Arch. Biochem. Biophys.* **50**, 148.
9) Laki, K. (1957). *J. Cell. and Comp. Physiol.* **49**, 249.
10) Kominz, D. R., Saad, F., Gladner, J. A. & Laki, K. (1957). *Arch. Biochem. Biophys.* **70**, 16.
11) Sheng, P. K. & Tsao, T. C. (1954). *Acta Physiol. Sinica* **19**, 203.
12) Tsao, T. C., Tan, P. H. & Peng, C. M. (1955). *Ibid.* **19**, 389.
13) Sheng, P. K., Tsao, T. C. & Peng, C. M. (1956). *Ibid.* **20**, 151.
14) Jen, M. H. & Tsao, T. C. (1957). *Ibid.* **21**, 91.
15) Hamoir, G. (1950). *Biochem. J.* 48, 146.
16) ——— (1951). *Ibid.* **50**, 140.
17) ——— (1955). *Advances in protein chemistry* **10**, 249.
18) Kubo, S. (1957a). *Bull. Fac. Fish. Hokkaido Univ.* 8, 147.
19) ——— (1957b). *Ibid.* 8, 214.
20) Dingle, J. R. & Odense, P. H. (1959). *J. Fish. Res. Bd. Canada* **16**, 53.
21) Saad, F., Kominz, D. R. & Laki K. (1959). *J. Biol. Chem.* **234**, 551.
22) Yoshimura, K. (1955). *Mem. Fac. Fish. Hokkaido Univ.* **3**, 159.
23) Bailey, K. (1956a). *Biochem. J.* **64**, 9 p.
24) ——— (1956b). *Pubbl. Staz. Zool. Napoli* **29**, 96.
25) Kominz, D. R., Saad, F. & Laki, K. (1957). *Nature* **179**, 206.
26) Laki, K. (1957). *Arch. Biochem. Biophys.* **67**, 240.
27) Bailey, K. (1657). *Biochim. et Biophys. Acta* **24**, 612.
28) Hanson, J., Lowy, J., Huxley, H., Bailey, K., Kay, C. M. & Rüegg, J. C. (1957).

Nature 180, 1134.
29) Kominz, D. R., Saad, F. & Laki, K. (1958). *Proceeding of the Conference on the Chemistry of Muscular Contration.* [p. 66]. Tokyo; Igaku Shoin Ltd.
30) Kay, C. M. (1958). *Biochim.* et *Biophys. Acta* 27, 469.
31) Laki, K., Horváth, B. & Klatzo, I. (1958). *Ibid.* 28, 656.
32) Kay, C. M. & Bailey, K. (1959). *Ibid.* 31, 21.
33) Snellman, O. & Tenow, M. (1954). *Ibid.* 13, 199.
34) Tenow, M. & Snellman, O. (1954). *Ibid.* 15, 395.
35) Miller, G. L. & Miller, E. E. (1948). *Anal. Chem.* 20, 48.
36) Spies, J. R. & Chambers, D. C. (1949a). *Ibid.* 20, 30.
37) ——— & ——— (1949b). *Ibid.* 21, 1249.
38) Allen, R. J. L. (1940). *Biochem. J.* 34, 858.
39) Zimm, B. H. (1948). *J. Chem. Phys.* 16, 1099.
40) ——— & Stockmayer, W. H. (1949). *Ibid.* 17, 130.
41) Bailey, K. (1954). *The Proteins* (Neurath, H. & Bailey, K. editors) Vol. 2, part B, [p. 1021]. New York; Academic Press Inc.
42) Rees, M. W. (1946). *Biochem. J.* 40, 632.
43) Smith, E. L. & Stockell, A. (1954). *J. Biol. Chem.* 207, 504.
44) ———, ——— & Kimmel, J. R. (1954). *Ibid.* 207, 551.
45) Hirs, C. H., Stein, W. H. & Moore, S. (1954). *Ibid.* 211, 941.
46) Harfenist, E. J. (1953). *J. Am. Chem. Soc.* 75, 5528.
47) Moore, S. & Stein, W. H. (1951). *J. Biol. Chem.* 192, 663.
48) Narita, K. (1954). *Tanpakushitsu Kagaku* (The Chemistry of Protein) (Akabori, S. & Mizushima, S. editors) Vol. 1, [p. 201]. Tokyo; Kyoritsu Shuppan Co. Ltd.
49) Ceriotti, G. & Spandrio, L. (1957). *Biochem. J.* 66, 607.
50) Chinard, F. P. (1952). *J. Biol. Chem.* 199, 91.
51) Schram, E., Moore, S. & Bigwood, E. J. (1954). *Biochem. J.* 57, 33.
52) Bailey, K. (1937). *Ibid.* 31, 1406.
53) Sanger, F. (1945). *Ibid.* 39, 507.
54) Levy, A. L. (1954). *Nature* 174, 126.
55) Frankel-Conrat, H., Harries, J. I. & Levy, A. L. (1955). *Methods of Biochemical Analysis* (Glick, D. editors) Vol. 2, [p. 359]. New York; Intersience publ.
56) Sanger, F. (1949). *Biochem. J.* 45, 563.
57) Betteheim, F. R. (1955). *J. Biol. Chem.* 212, 235.
58) Akabori, S., Ohno, K., Ikenaka, T., Nagata, A. & Haruna, I. (1953). *Proc. Japan Acad.* 29, 561.
59) Ohno, K. (1953). *J. Biochem.* (*Tokyo*) 40, 621.
60) ——— (1954). *Ibid.* 41, 345.
61) ——— (1956). *Tanpakushitsu Kagaku* (The Chemistry of Protein) (Akabori, S. & Mizushima, S. editors) Vol. 4, [p. 209]. Tokyo; Kyoritsu Shuppan Co. Ltd.
62) Lowther, A. G. (1951). Nature 167, 767.
63) Narita, K. (1958). *Biochim. et Biophys. Acta* 28, 184.
64) Rüegg, J. C. (1958). *Biochem. J.* 69, 46p.
65) Eliott, F., Hanson, J. & Lowy, J. (1957). *Nature* 180, 1291.
66) Kahn, J. S. & Szent-Gyorgyi, A. G. (1959). *Science* 130, 160.

昭和36年 6 月15日　印　刷
昭和36年 6 月30日　発　行

北海道大学水産学部
函館市港町253番地

印刷所　株式会社　笠井出版印刷社
東京都港区芝南佐久間町1の53

MEMOIRS
OF THE
FACULTY OF FISHERIES
HOKKAIDO UNIVERSITY